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STUDIES ON THE CENTRAL AND PERIPHERAL NERVOUS SYSTEMS OF TWO POLYCHÆTE ANNELIDS.

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I. INTRODUCTION.

My study of the nervous system of certain annelids was begun in the summer of 1895 at Wood's Hole, at the suggestion of Prof. C. O. Whitman, who proposed that I should if possible ascertain the nature of the metamerism of Clymenella torquata, as expressed in its nervous system. This species proved to be poorly adapted to the elucidation of this question; but the investigation of the nervous system has been continued during my two years of study at Radcliffe College, under the direction of Professor Mark of Harvard University. The work undertaken at Wood's Hole was confined to Clymenella torquata, but the discovery of a new worm, Clymene producta, and the use of special methods of preservation, furnished me the material that first suggested an investigation of the so called giant fibres and giant cells. Sections of this worm killed in vom Rath's fluid gave me remarkably satisfactory preparations of many parts of the nervous system, especially of the giant cells and the sensory organs.

The two polychæte annelids with which this paper deals are members of the family Maldanidæ. One, Axiothea * (= Clymenella) torquata, is found in the sand flats of many regions of our New England coast. The other belongs to a different genus. It is a new species, which I found in one of the small harbors opening into Vineyard Sound. I have found it in only this one locality, although I have examined the sand flats of many parts of the shore. Its distribution would seem, therefore, much more limited than that of Axiothea torquata. In a recent description (Lewis, '97) of this new worm, I have proposed for it the name Clymene producta, the specific name having been chosen on account of the great length of the worm. It is sufficient here to state that this worm can be readily distinguished from Axiothea torquata by the much greater number of segments which it possesses, and by a series of red bands upon the anterior abdominal segments.

The nervous systems of these two worms, both the central system and the sense organs, present many interesting features, the description of which must be omitted from the present paper; such, for instance, as the eyes of the new species, and in case of both worms the ciliated grooves of the head.

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* As I have shown in a recent paper (Lewis, '97), there is not sufficient ground for retaining the generic name Clymenella, proposed by Verrill, since the worm described under that name clearly belongs to the genus Axiothea of Malmgren.

In spite of the great amount of productive work which has been done in recent years on nerve anatomy and histology, many of the prominent questions connected with nervous structures are still matters of dispute. The neuron theory, the question of the restriction of medullated nerve fibres to vertebrates, the nature and function of giant fibres in invertebrates, the relation of sense organs to nerve fibres, and, very recently, the presence and meaning of a centrosome in ganglionic cells, — all these are questions of a broad and fundamental nature, and every observation, however limited, which bears directly upon the solution of any of these questions is of value to those engaged in the study of nervous structures. A very few weeks of study upon the nervous system of the two annelids named demonstrated that they were peculiarly interesting in relation to some of these particular questions; for this reason my work has been restricted to observations bearing upon these questions of neurology.

II. CENTRAL NERVOUS SYSTEM.

The topics treated under the Central Nervous System fall naturally into two divisions, The Leydig's Fibres, and The Giant Cells. But for a correct understanding of these structures a short general account of the nervous system will be necessary.

1. General Topography.

a. *Location of Nervous System.*

The whole nervous system in these annelids seems to present a very rudimentary condition. With the exception of the brain, it lies in the hypodermis throughout the whole length of the animal. At no region except the brain is it found internal to the circular muscles. The ganglionic cells are always distinguishable from the adjacent cells of the hypodermis by their form and nuclei; but on the ventral side of the nerve cord there is no membrane nor other boundary separating them from the adjacent cells of the hypodermis (Plate **1**, Fig. 5, Plate **3**, Fig. 21). On the dorsal surface of the nerve cord, however, and on the dorsal parts of its lateral edges, such a boundary is present. The circular muscles on the dorsal side of the cord are everywhere strongly developed, and the lateral nerves pass off ventral to these muscles.

b. *Distribution of Ganglion Cells.*

There is no evidence of any strictly segmental arrangement of the parts of the central nervous system, either in the grouping of cells into

ganglia, or in the relationships of the nerves given off from the ventral cord. Ganglionic cells are found abundantly throughout the length of the central nervous system, but, while more numerous in some regions than in others, they show no metameric plan of arrangement, nor any definite grouping into ganglia.

c. *Number and Distribution of Nerves.*

In each body segment lateral nerves are given off from the ventral cord in very large numbers. In some segments I have demonstrated more than thirty-five on each side of the body. The only feature of these nerves in any way suggestive of a metameric arrangement is their greater size in the region of the parapodia. These nerves are without a sheath, branch freely, and anastomose with one another, so that beneath the hypodermis there is a network of nerve fibres which forms an almost continuous sheet (Plate **1**, Fig. 4). Figures 66 and 67 (Plate **8**) are diagrams obtained by superimposing in each case a series of frontal sections of C. producta. Each shows the number and arrangement of nerves and giant cells in the anterior part of one segment. Figure 21 (Plate **3**) is a typical cross section of the nerve cord (from C. producta); it shows that the ganglionic cells occupy the ventral and lateral portions of the cord, and that on the dorsal side of the cord there is the cross section of what appears to be a tube. This is Leydig's fibre, which lies just ventral to the circular muscles.

In general, these facts point to an undifferentiated condition of the nervous system. Whether this condition is primitive, or has arisen secondarily, and therefore represents a degraded state of the nervous system, can of course be settled only by the aid of phylogenetic as well as ontogenetic evidence.

2. Methods.

My studies on the central nervous system of these annelids had to be pursued almost entirely upon sections of hardened material. Attempts to isolate the nerve cord were very unsatisfactory, because of the close intimacy of nervous system and hypodermis. Several methods of fixing and staining were employed, but of these the one given by vom Rath ('93, p. 102) proved to be pre-eminently valuable for the study of every part of the nervous system.

To prevent undue contraction it was found necessary to narcotize the worms thoroughly in all cases before killing. For the nervous system, owing to its situation in the hypodermis, partakes of all the folds of the

body wall resulting from the contraction of the animal. A narcotizing mixture of about 5 volumes of 95% alcohol to 95 volumes of sea water gave the best results. A fuller account of the methods employed for studying different structures will be given under the separate topics.

3. Leydig's Fibres.

I have selected the name Leydig's fibre for a long known structure in annelids described under several different names; the principal ones, besides that which I have chosen, are neurochord, giant fibre, central canal, and neural canal. The name Leydig's fibre is adopted because Leydig was the first writer to give an accurate description of this structure; he also ascribes to it the nature which the most thorough and careful of recent investigations shows to be the most probable.

a. *Historical Review.*

These fibres have been studied by many writers, and the literature upon them is extensive. Bibliographical lists of considerable length, arranged chronologically, have been given by Spengel ('81, p. 41), Eisig ('87, p. 476), and Friedlaender ('89, p. 206; '94, p. 662).

But in spite of the fact that so much attention has been given to these structures, the most widely divergent conclusions have been drawn regarding their morphology and function, the latest papers upon the subject being no more harmonious than were the earlier ones. At least five different theories regarding the Leydig's fibres in annelids have been strongly urged.

First, that they are true nerve fibres of large size. This view was first set forth by Leydig in 1864, and it has been consistently supported by him in several subsequent papers. He declares these structures to be large medullated nerve fibres, — "riesige, dunkelrandige Nervenfasern," — and regards them as similar to the medullated nerve fibres of vertebrates. This theory received confirmation from Spengel ('81), who was the first to show that these fibres were the processes of ganglionic cells. Since, in the case of the annelid which he studied the number of longitudinal fibres remains tolerably constant and is much less than the number of giant cells, he believes that the larger fibres — "grösseren Röhren" — have arisen through the union of the direct processes of these ganglion cells.

Secondly, the view defended by Claparède (first in 1862, and several times subsequently), according to which these so called fibres have the nature of canals. While the various authors who use the terms canal or

tube differ much in regard to the details of structure, they agree in the one notion, that Leydig's fibres are tubes. Most of the advocates of this view ascribe to these fibres no definite function.

Thirdly, that advanced by Kowalevsky ('71), which makes these structures supporting organs, homologous to the chorda dorsalis of vertebrates, and denies to them any nervous nature whatever.

Fourthly, the view advocated by Vejdovský ('84), which maintains the supporting nature of Leydig's fibres, but denies to them any genetic relationship to the chorda dorsalis of vertebrates. The relationship is one of analogy alone, since Leydig's fibres are derived from mesoderm, whereas the chorda dorsalis of vertebrates comes from entoderm. This view agrees with the third in denying all nervous nature to Leydig's fibres.

Fifthly, the idea advanced by Eisig ('87), which is that Leydig's fibres and the giant cells connected with them are in the young stages of the worm nervous in nature, but that later they undergo degeneration, and that the fibres finally come to function as a supporting organ. Thus they may in the adult worm be compared functionally with the chorda. He further suggests a comparison of these structures with the white matter of the nervous system of vertebrates, saying ('87, p. 483), "Auf Eine von mir schon im Vorhergehenden betonte Thatsache möchte ich aber bei dieser Frage nach der Bedeutung der Neurochordnerven noch einmal zurückkommen, weil sie möglicherweise mit zum besseren Verständnisse beitragen kann: ich meine die Thatsache, dass wir *im Nervensysteme der Anneliden fortan zwei Bestandtheile zu unterscheiden haben. Den einen bildete das dauernde, aus feinsten Fibrillen und zahlreichen kleinen Ganglienzellen sich aufbauende System, den anderen bildet das allmählich der Degeneration unterliegende, aus breiten Nervenfasern und wenigen riesigen Ganglienzellen zusammengesetzte.* Die Elemente des ersteren Bestandttheiles wurden in Anbetracht ihres histologischen Verhaltens öfters der 'grauen Substanz' der Vertebraten-Centren verglichen — vielleicht dürfen wir diejenigen des letzteren der 'weissen Substanz' gegenüberstellen."

Finally, there are those who deny to Leydig's fibres any nervous nature, on the ground that these fibres do not react to the Golgi method. These writers do not commit themselves to any definite statement as to the function of the structures in question.

The following lists give, in chronological order, the chief supporters of these various theories: —

1. Giant nerve fibres; truly nervous in nature.
   '64, '64[a]. Leydig.
   '78. Schultze.
   '80. Langerhans.
   '81. Spengel.
   '83. Vignal.
   '83. Jacoby.
   '86. Leydig.
   '87. Rohde.
   '88. Friedlaender.
   '89–'91. Hatschek.
   '89. Friedlaender.
   '92. Cerfontaine.
   '94. Friedlaender.
   '95. Friedlaender.
2. Canal nature; axial canal or central canal.
   '62. Claparède.
   '62[a]. Claparède.
   '62. Keferstein.
   '63. Clarapède.
   '64–'68. Ehlers.
   '68. Claparède.
   '69[a]. Claparède.
   '73. Claparède.
   '74. Greff.
   '76. Semper.
   '78. Vejdovský.
   '78. McIntosh.
   '79. Vejdovský.
   '80. Spengel.
3. Homologous to chorda dorsalis of vertebrates.
   '71. Kowalevsky.
   '74. Semper.
   '81. Perrier.
   '85. Cunningham.
4. Analogous to chorda, but not related genetically.
   '82. Vejdovský.
   '83. Bülow.
5. Degenerate nerve fibres; the sheath or tube-wall retained as an organ of support.
   '87. Eisig.
   '88–'94. Lang (p. 221).
6. Not nervous in nature because they do not respond to the Golgi method.
   '92. Von Lenhossék.
   '92–92[a]. Retzius.

The latest work, and the most conclusive of all, upon the Leydig's fibres, is that by Benedict Friedlaender. His observations and conclusions are based upon a very broad and complete series of investigations, — upon careful physiological experiments, as well as comparative morphological study. After investigating thoroughly both sheath and contents, his conclusion is, that Leydig's fibres are true nerve fibres arising from ganglionic cells, and further that they are *medullated* nerve fibres of the type found in vertebrates; that the sheath agrees in structure, at least in great part, with the myelin sheath of nerves in vertebrates, and that the contents represent the axis cylinder.

As his later papers are in full agreement with his earlier article of 1889, the words of the latter ('89, p. 258) may be quoted here: "Die sogenannte Neurochorde von Mastobranchus, Lumbricus und sehr wahrscheinlich die der Anneliden überhaupt, . . . endlich die markhaltigen Fasern der Wirbelthiere sind fundamental dieselben Gebilde."

b. *Methods employed.*

The present observations upon the fibres of Leydig were made upon sections only. These have been prepared from tissues fixed and stained by several methods. The reagent which proved most satisfactory for both contents and sheath was the picro-osmic-acetic-platinic chloride mixture of vom Rath ('95, p. 282), followed by wood vinegar. To obtain good preparations, it was very important to allow the material to remain for a long time in alcohol before embedding. Such preparations were valuable for tracing the courses of the cell-processes entering Leydig's fibres, as the sheath was stained black and therefore stood out prominently against the clear gray contents of the fibres and the surrounding nervous tissue. In preparations obtained by this method the course of the cell-processes in entering Leydig's fibre could be traced with low powers of the microscope. Other material, fixed in corrosive sublimate, alcohol, or formol, and stained with ordinary hæmatoxylin dyes or with iron hæmatoxylin, were used for comparison.

c. *Structure of Leydig's Fibres.*

Leydig's fibres from specimens of both C. producta and A. torquata, killed in the vom Rath mixture, differed considerably in the appearance of both the contents and the sheath from those prepared by other means. In sections of material prepared by the vom Rath method the sheath of the fibre was much more prominent than in those obtained by other methods. It appeared sometimes as a single wall with double contour, but more often gave the appearance of being composed of strands or lamellæ (Plate **2**, Figs. 15, 16). It becomes black by this treatment, and is readily distinguishable from the neuroglia, which stains brown, and also from all other parts of the nervous system. In this blackening with osmic acid it shows an agreement with the medullary sheath of the nerve fibres of vertebrates. On the other hand, in sections prepared from alcoholic or sublimate material, the sheath, if it could now be called such, was often inconspicuous, and in places seemed to be lacking entirely. Sometimes after such treatment it gave the appearance of strands or lamellæ, but it never exhibited the prominent condition which preparations of the vom Rath material showed. This changed appearance of the sheath I believe to be due to its having been partly dissolved by the alcohol, thus leaving in the sections only a part of the original sheath.

The contents of the fibre in most of the vom Rath material entirely filled the sheath, but occasionally they were slightly shrunken away from

the wall. The contents were pale, clear, bluish gray in color, and presented a very delicate structure, which is uniform throughout the fibre. Leydig's fibre consists, therefore, of a single nerve fibre, not of a bundle of fibres. I have not been able to discover by any of the methods used anything in the contents of a fibrillar or striate nature. In a few cases it seemed as if the granules showed for a short distance a linear arrangement, but not such a condition as could properly be called fibrillar. In sections prepared by ordinary methods of fixing, these fibres often showed shrinkage; and such appearance might oftentimes lead to the conclusion which Eisig drew in the case of the Capitellidæ, viz. that the fibres showed evidence of degeneration.

It is clear, then, that the results here given regarding the finer structure of Leydig's fibres in the case of Clymene producta and Axiothea torquata agree in almost all respects with those of Friedlaender. The conclusions of the present paper are: —

1) Leydig's fibre is a tubular structure, consisting of wall and contents. The wall blackens intensely when treated with osmic acid, agreeing in this respect with the myelin sheath of the nerve fibres in vertebrates. In alcoholic preparations the sheath partially or entirely disappears.

2) The contents of the tube show an exceedingly fine and delicate structural condition, identical with that of the individual nerve processes of ganglionic cells. In sections prepared by the vom Rath method, they present the appearance shown in Figure 16 (Plate **2**). There is not the slightest evidence that Leydig's fibre is composed of a bundle of nerve fibrillæ.

d. *Relation to Cells.*

As far as regards the topographical relation of Leydig's fibres to the cells connected with them, these two Maldanidæ differ markedly from all other annelids in which these structures have been described.

In all the accounts which I have read of giant cells and connected giant fibres, the cells have been described as showing a metameric distribution. But in the cases of both C. producta and A. torquata, I find no evidence of a metameric arrangement of the giant cells. In both worms the cells which give rise to the giant fibres are situated without any discoverable regularity or symmetry along the sides of the nerve cord, and in a few instances ventral to it. The number of such cells varies in different segments. The giant cells of the two species correspond in position only in so far as there is no regular plan of distribution in either case. In both instances they present a well marked and charac-

teristic cell-structure, which serves to distinguish them from the other cells of the nervous system. This structure will be described later, after a description and comparison of the courses of the fibres in the two species has been given.

The processes of the different giant cells, after a more or less irregular course, enter the Leydig's fibre, and immediately upon their union with it the individual processes lose their respective sheaths. The contents of the process, fusing with the contents of the Leydig's fibre, lose their identity. Such a connection of fibre and cells has been demonstrated in dozens of my preparations. Several times a single fortunate section has shown the connection of cell and fibre, but far more often the connection was established only by following through several successive sections. *A large number of absolute demonstrations in various parts of the worm leave no doubt that the Leydig's fibres are continually receiving the processes of certain large and peculiar cells,* occurring in very restricted number in the midst of the smaller cells of the nervous system (Plate **4**, Figs. 22, 23).

The Leydig's fibre is single and median in position through the whole thoracic region of both worms; but in the sub-œsophageal ganglion it divides into two symmetrical arms, which diverge and apparently end in a pair of symmetrically placed giant cells. I have not been able to determine with absolute certainty the nature of the anterior termination of these arms, for I have not ascertained whether the anterior end of Leydig's fibre always terminates in giant cells, or whether it may extend forward of the most anterior cell-processes with which I find it connected. My reason for believing that it terminates in a pair of giant cells is, that in the same section in which the most anterior part of the paired portion of the fibre is met with, or in sections very near to it, there is also found the anterior pair of giant cells. Sometimes these are clearly connected with the corresponding branches of the fibre, but the evidence on this point is not always satisfactory, and in some cases it seems possible that the branches of the fibre may end independently of the first giant cells.

In one important respect the results here presented regarding these cell-processes and Leydig's fibre differ from those of Friedlaender, viz. that no branching or anastomosing of the cell processes or of Leydig's fibre has been observed, and I believe that no such branching occurs.

e. *Comparison of the Course of Leydig's Fibres in the two Worms.*

In Clymene producta the conditions in the first twenty segments have been studied. Although the worm probably consists of more than

seventy segments, the difficulty of obtaining entire worms has prevented such a study of the remaining segments as would give reliable topographical results. In these twenty segments I find the following conditions. Leydig's fibre appears in the sub-œsophageal ganglion as a pair of tubes which, passing caudad, very soon unite to form a single fibre extending backward along the mid-dorsal region of the nerve cord. It increases somewhat in diameter as it runs caudad, but the increase is slight in proportion to the size and number of the cell-processes which enter it. In this worm there is to be found in the first twenty segments only one Leydig's fibre.

In Axiothea torquata the fibre of Leydig consists in the sub-œsophageal ganglion of two tubes, precisely as in C. producta, as is shown by a series of cross sections of the worm through this region. These two tubes in passing caudad soon unite, thus producing a single fibre, which extends backward along the mid-dorsal region of the nerve cord as in C. producta. But this fibre continues single only as far as through the seventh segment. Beyond the seventh segment there are two fibres lying side by side almost in contact, and extending as far as the nineteenth segment. In the nineteenth segment the two again unite, and from this point onward there is again only one Leydig's fibre; this continues undivided, and ends in the twenty-second segment.

As long as Leydig's fibre remains a single structure, it shows the same relation to the giant cells as in the case of Clymene producta; but in the region where there are two fibres side by side, I find that some of the giant cells send their process into the fibre of the corresponding side of the cord, whereas the process of others crosses the median plane to enter the more remote fibre.

The two Leydig's fibres of Axiothea torquata extend throughout most of their course parallel to each other, lying side by side, but in certain places they cross each other. The crossing may be a single isolated one, or there may be two crossings in quick succession, so that an exchange of positions is quickly followed by a return of both to their original relations to the body. In these successive crossings, whether close together or far apart, a fibre, first passing above (dorsad of) its mate, may then pass back to the side on which it originally lay, either dorsad or ventrad of the other fibre. The first condition results in a simple crossing, the second in a real twisting of the fibres. In either crossing or twisting no anastomosing or branching of the two fibres has been discovered.

While the crossings of Leydig's fibres show in the different segments considerable variation in plan and number, there seems always to be at

least one crossing near the parapodia; in no case have more than five crossings been found in a single segment. Thus it seems that in regions where two fibres exist side by side they show more evidence of metamerism than does the rest of the nervous system.

In the posterior segments of the worm it is difficult to obtain single sections that show any considerable length of Leydig's fibres. The whole nervous system follows the course of the epidermis, which in these segments is thrown into small folds or convolutions, whatever be the method employed in killing.

Attention may here be called to the fact, that in both these Maldanidæ the central nervous system lies external to the ring muscles, which serve as a mechanical support to it. In these cases, therefore, it is probable that Leydig's fibres do not themselves fulfil the same function.

f. *Function of Leydig's Fibres.*

That Leydig's fibres are truly nervous in nature is supported, first, by their direct connection with ganglion cells located in the midst of the central nervous system; secondly, by the finer structure of the fibre, both sheath and contents; and, thirdly, by the position of the fibres in the central nervous system.

The cells with which these fibres are connected certainly lie within the territory of the central nervous system, and are surrounded by other nerve cells. Their processes are traceable among the fibres of the ventral cord until they reach the Leydig's fibre. Such cells are found nowhere else in the animal except in the region of the nervous system. They show a marked resemblance in certain very important and peculiar details of their internal structure to the ganglionic cells described by von Lenhossék ('95[a]) for the central nervous system of the frog. These points of resemblance will be discussed later, when a detailed description of the cells is given.

It would seem as though the evidence that the cells in question are of a nervous nature was almost beyond question; but if this is so, then Leydig's fibres, which clearly are in direct continuity with the processes of the cells must likewise be of a nervous character. So far as I know, those writers who have themselves demonstrated the continuity of cells with Leydig's fibres have not denied the nervous nature of these structures. Only a very few of all the writers upon this subject, it is true, have actually shown such a connection. These authors are Spengel ('81), Rohde ('87), Haller ('89), Friedlaender ('89), and Cerfontaine ('92), all of whom are supporters of the nervous nature of Leydig's fibres.

Eisig, who believes that such continuity exists, although he did not actually demonstrate it, grants that the structures were originally nervous in nature, although in his opinion they have since become degenerate.

The ground upon which their nervous nature is denied by von Lenhossék and Retzius — viz. their failure to react to the Golgi method — seems very unimportant, especially when compared with the satisfactory work and positive results obtained by Cerfontaine with methylen blue. The capricious nature of the Golgi method is well known. It is granted by all workers who have employed this method that there are many nervous structures in any preparation which do not react to the silver process. Indeed, one of the things which makes a Golgi preparation especially valuable is the fact that only a small proportion of the nervous elements are affected by this treatment. Considering the exceedingly small number of Leydig's fibres in any preparation, and the limited number of workers who have employed the Golgi method upon them, it is not strange that no reaction has yet been obtained.

The evidence, however, is not yet sufficient to allow one to draw a safe conclusion as to the particular office of Leydig's fibres in the nervous economy of the worm.

g. *Bearing of the Condition of Leydig's Fibres in Annelids upon the Neuron Theory.*

The conditions of Leydig's fibres in Clymene producta and Axiothea torquata, as I have described them, and especially their relations to the great ganglion cells, have an important bearing on the recent neuron theory. We have here dozens of nerve cells sending their processes into the same nerve fibre. Immediately upon entering this fibre, these processes lose their respective sheaths, and to all appearances fuse with the contents of the fibre, thus losing their individuality. These facts are not easily reconciled with a theory which maintains that the relation of the processes of one nerve cell to those of another nerve cell is that of contiguity alone.

The neuron theory is now several years old, and it seems a little strange that when the generalization was extended to include the nervous system of invertebrates, no account was taken of the difficulty presented by Leydig's fibres, although the relation of such fibres to ganglionic cells had long been described. It was Waldeyer ('91) who first emphasized the principle of the nerve unit; and introduced the term "Neuron." He says (p. 52 of Separate): "Das Nervensystem besteht aus zahlreichen intereinander anatomisch wie genetisch nicht zusammenhängenden Nervenein-

heiten (Neuronen)." Von Lenhossék ('95) declares the principle of the neuron to be true for invertebrates as well as vertebrates. He says ('95, p. 108): "Auch bei den wirbellosen Tieren setzt sich das Nervensystem aus Neuren zusammen." Later (p. 109) he says: "Nervenfaser, Terminalverästelung und Seitenzweige stellen leitende Medien, Auswüchse des Zellkörpers dar, die dieser dem Bedürfnisse entsprechend entwickelt, zu nahe und entfernt von ihm gelegenen Elementen Beziehungen einzugehen: andere Nervenzellen zu umspinnen, in sensible Endbezirke hineinzuragen oder sich an kontraktile Elemente anzulöten. Diese *Beziehungen bestehen stets in einem innigen Kontakt.* Darin liegt ein *wichtiges Organisationsgesetz,* nicht nur für die höheren Lebewesen, sondern auch für die wirbellosen Tiere, bis zu der Lebensstufe hinunter, wo die erste Nervenzelle und Nervenfaser in die Erscheinung tritt."

But in the case of Leydig's fibre, the entire weight of evidence is to the effect that we have direct continuity of substance, not simply intimate contact, between the processes of different nerve cells. I am aware that a zealous supporter of the neuron theory might claim that, although the individual cell-processes lost their respective sheaths on entering Leydig's fibre, still each process remained distinct and maintained its individuality, — that Leydig's fibre was simply a bundle of distinct nerve processes extending side by side, but closely pressed together. The evidence offered by the work of Cerfontaine ('92) with methylen blue is, however, entirely against such a supposition. Every worker with methylen blue as an *intra vitam* stain knows its tendency to pick out and stain a few nerve cells and nerve fibres. But the results obtained by Cerfontaine gave no evidence that parts of the fibre were selected by this stain and others left unstained, as would almost certainly be the case if it were composed of physiologically distinct strands or tracts. His figures show that Leydig's fibre, when treated with methylen blue, is a single homogeneous structure.

I regret that I have not had time and opportunity to experiment with the action of methylen blue upon these structures. But I believe that the facts already presented prove that there is no separation or boundary between these particular cell processes. But if there is no discoverable boundary, it is highly improbable that the processes retain their individuality and physiological independence. Leydig's fibre seems to be an effective means of bringing about an intermingling of the substance of these processes. The anastomosing of the different Leydig's fibres which Friedlaender and Cerfontaine describe in case of Lumbricus is another condition which harmonizes with this interpretation.

In view of these facts, we are led to believe that, whether or not the neuron theory holds true for vertebrates, it certainly does not apply to all invertebrates.

4. Giant Cells.

a. *General Account of Size, Number, Arrangement, etc.*

Under the name giant cells I include those large cells whose processes unite to form Leydig's fibre. In both worms the most anterior of these cells occupy the sub-œsophageal ganglion; and they are found in small numbers throughout the ventral cord. They are usually situated in the lateral portions of the nerve cord, but sometimes lie in the ventral part of it. They are not found in the brain. While in the larger number of cases the connection of these cells with the Leydig's fibre could not be shown in a single section, yet in several cases it has been possible to do this. It is not strange, in view of the frequently curved and irregular course of the cell-process, that such fortunate sections should be obtained only occasionally.

As has been stated before, these cells show no symmetry or regularity of arrangement, except in the region of the sub-œsophageal ganglion. In all the specimens examined, the giant cells of this region seemed to a certain extent to show a paired arrangement. In no other portion of the cord has anything approaching symmetry been found.

In neither species do the different segments of the worm show agreement in the number of these cells. By superimposing drawings of serial sections of two segments, I found the number of the cells in two successive segments of the nephridial region to be in one case twelve, in another eight.

The dimensions of the cells vary, and are in a measure proportional to the diameter of the worm, for they increase in size from its anterior end as far as that portion of the body in which the nephridia are found, this being the thickest part of the worm, and from this region onward they again diminish in size. The cells are also most numerous in the segments bearing nephridia, becoming scantier farther behind. But even in that part of the body where the cells are largest, they vary considerably in size. A comparison of several cells from the nephridial region gave a variation of from 30μ to 52μ in the long diameter, and of from 20μ to 40μ in the short diameter. Although showing this considerable variation in size, any of these cells are large enough to be easily distinguished from other cells of the nervous system, even by the use of very low powers of the microscope.

The giant cells are all unipolar. At least I have never found more than one nerve process, and I do not believe that more than one exists. In entering the fibre of Leydig the cell-process almost always takes a backward direction. Its course from the cell may be for some distance forward, but before entering the fibre of Leydig it is usually found to turn caudad (Plate **4**, Fig. 23). In a very few instances, in the posterior segments of Axiothea torquata, it has been seen to be directed cephalad as it entered the fibre.

The giant cells and their processes are surrounded by the same sort of a sheath as that which has been described for Leydig's fibre. It is evidently one continuous sheath extending around cell, cell-process, and Leydig's fibre. Around the cells it shows itself to be composed of several strands or lamellæ. The process from the giant cell shows an internal structure identical with that of Leydig's fibre, so that the account of the internal structure of the Leydig's fibre already given can be applied without change to the individual cell-processes. In sections which show the union of cell-process and Leydig's fibre there is, so far as the appearance of the contents of both go, no mark by which one could be distinguished from the other. Sections prepared from material treated with the vom Rath mixture show a delicate gray finely granular protoplasm which entirely fills the sheath (Plate **2**, Figs. 15 and 16) like that which has been described for the Leydig's fibre.

b. *Technique.*

The internal structure of the cell itself shows peculiar conditions, which are constant and equally well shown by two entirely different methods of fixation and staining; viz. (1) the vom Rath mixture already described, followed by wood vinegar, and (2) a cold saturated aqueous solution of corrosive sublimate followed by iron hæmatoxylin as a stain. The first method was in some respects the more satisfactory, since it seldom showed any shrinkage of the cell contents. But the iron-hæmatoxylin stain employed upon sections fixed with corrosive sublimate could be controlled more easily than the vom Rath preparations. The principal results regarding the internal structure of the cell obtained by one method were, however, fully confirmed by the other. It was very important in the use of the vom Rath mixture that there should not be too much osmic acid in the solution, and also that the material, after being treated with the wood vinegar, should remain for several days, or better weeks, in strong alcohol.

c. *Minute Structure.*

The giant cells are never spherical, their axes being of unequal length (Plate **1**, Figs. 1, 2, 3; Plate **2**, Figs. 8, 10, 13). Sections through the long axis of the cell are very unlike those through the short axes. In a section made parallel to the long axis, the nucleus is seen to occupy an eccentric position (Figs. 1, 2, 3), and is sometimes found lying in a sort of outpocketing of the cell (Plate **2**, Fig. 10). The nucleus, although large and distinct, is relatively much smaller than the nuclei of the smaller cells of the nerve cord (Plate **3**, Fig. 21), and has a well marked nuclear membrane. It usually contains only one large nucleolus, but sometimes two. The axes of the nucleus are frequently unequal, measurements of a dozen or more nuclei having given a variation from 11μ to 20μ for the long diameter, and from 8μ to 12μ for the short diameter. But sometimes the nucleus appears round, or nearly so, when cut in the long axis of the cell, — the direction which shows the inequality of the nuclear axes, if any exist. In sections perpendicular to the long axis of the cell the nucleus occupies a central position.

Near the nucleus, but nearer the centre of the cell, is a peculiar structure which seems as constantly present as the nucleus itself. In none of the giant cells prepared by either of the methods given was it ever wanting, although the details of the structure were not altogether uniform. The new species, Clymene producta, was much the more favorable of the two for the study of this structure, and all the giant cells figured are from that worm. No similar body has been mentioned for giant cells in other annelids, although the giant cells themselves have been described in the nervous system of annelids many times. An article by von Lenhossék ('95[a]), which was received shortly after I first discovered these cells, describes for the nerve cells of the frog, under the name centrosome and sphere, a structure which resembled so strongly the peculiar body in the giant nerve cells of Clymene producta, that there could be no doubt of their being identical.

For the present, I shall designate the entire structure by the name "sphere," and later discuss the application of the term.

In the case of these giant nerve cells there could be made out in the sphere the following well marked regions. (Plate **1**, Figs. 2, 3; Plate **2**, Figs. 11, 12; Plate **4**, Figs. 24, 26; Plate **5**, Figs. 29–32.)

(1) The outer part of the differentiated region consists of a broad zone of rather coarse granules; (2) within this is a smaller central area of nearly homogeneous protoplasm; and (3) within this central area a

minute deeply staining body, or sometimes three or four such bodies. This corpuscle or group of corpuscles I call centrosome, employing von Lenhossék's term, although in the structure so designated there is a lack of agreement with von Lenhossék's centrosome, which will be pointed out later. There are radiations, which sometimes extend out beyond the sphere into the undifferentiated portions of the cell, but sometimes only through the coarsely granular zone of the sphere. In some preparations (Plate **1**, Fig. 2), these radiations traverse both zones, and seem to take their origin from the central corpuscle. In others (Plate **4**, Fig. 26), the radiations traverse only the outer more coarsely granular zone, and do not cross the inner more homogeneous area. The rays were usually present in rather small numbers, so that they could be readily counted. In such cases, they were generally separated by nearly uniform intervals, although often they were interrupted over an arc of many degrees. Occasionally the radiations were too numerous to be readily counted. In several preparations, they were so clear and strong that they could be seen without the use of anything higher than an E (Zeiss) objective, but in others they were brought out only by the use of an immersion lens. A ring of microsomes appeared in one preparation at a uniform distance from the central corpuscle (Plate **2**, Fig. 11).

The sphere was sometimes rather sharply marked off from the remaining protoplasm of the cell, but usually graduated into it.

In all the specimens of Clymene producta studied, there were found in the regions of the sub-œsophageal ganglion, and nowhere else, giant cells which contained each two spheres (Plate **2**, Figs. 8, 9, 13, 14). It was sometimes possible to make out in these cells a central corpuscle and radiations, but not with the same clearness as in many of the cells containing a single sphere. In most instances of cells containing two spheres, the spheres are very sharply marked off from the rest of the cell protoplasm, sometimes by a ring of granules, sometimes by a clear space around the sphere. It seems to me a peculiar fact that cells containing two spheres should always be found in this region and not in other parts of the worm; but I have no explanation of this fact to offer.

Outside the sphere the contents of these giant cells consist of a clear protoplasm, which appears identical with that of the cell-process and Leydig's fibre. In many cells there is at a little distance from the sphere a band of denser protoplasm, which partially surrounds the sphere, but is interrupted on the side toward the nucleus, apparently owing to the presence of that structure.

By the vom Rath method, as well as by the use of iron hæmatoxylin,

the central corpuscle stains much more intensely than the surrounding zone. Occasionally it is slightly irregular in shape, and, as before stated, sometimes gives the appearance of a single body (Plate **1**, Fig. 2), but more often there seemed to be present several — two, three, or four — exceedingly small corpuscles (Plate **4**, Fig. 24).

Vacuoles resembling those described by Rohde and other writers sometimes occurred in the protoplasm close to the sheath surrounding the cell (Plate **1**, Fig. 3; Plate **4**, Fig. 25; Plate **5**, Fig. 29).

It is not difficult to trace a single giant cell through all the sections in which it is embraced, because this peculiar type of cell occurs so rarely in the nervous system that two such cells would seldom, perhaps never, occur so close together as to permit their being mistaken for each other.

d. Centrosome.

(1) *Historical Review.*

It is only recently that centrosomes were first recognized in nerve cells, and for that reason, if for no other, they have been mentioned by only a very few writers. So late as 1894, when Heidenhain wrote his paper, "Neue Untersuchungen über die Centralkörper und ihre Beziehungen zum Kern- und Zellenprotoplasma," such a structure was not known to exist in nerve cells. Heidenhain ('94, p. 656) merely suggests the possibility of its presence, for he closes his chapter regarding the universality of the centrosome with these words: "Kämen wir so zu positiven Resultaten, so würde es am Ende der van Beneden-Boverischen Theorie von der Ubiquität der Centralkörper am Ende nur einen geringen Eintrag thun, wenn sich schliesslich herausstellen sollte, dass es einige wenige Zellenformen giebt, welche, da sie im erwachsenen Thierkörper nicht mehr theilungsfähig sind, die Centralkörper völlig einbüssen. Es würde sich, soweit ich das zu beurtheilen vermag, wohl wesentlich nur um die Ganglienzellen handeln. Allein auch hier ist für eine Specialuntersuchung Aussicht auf Erfolg vorhanden, da doch von einigen Ganglienzellenformen her sowohl radiäre als auch concentrische Anordnungen bekannt sind."

Von Lenhossék ('95[a]) was the first author positively to demonstrate the presence of a centrosome in ganglion cells. He describes very fully for certain of the spinal ganglion cells of the frog a well defined centrosome and sphere, and gives a detailed account of all the parts of the structure. As von Lenhossék was the first author to discover and describe this structure in nerve cells, I have preferred to make use of his terms "centrosome" and "sphere," although in some respects the conditions in the

cells which I describe agree best with Heidenhain's "Microcenter" and "Centralkörper." Von Lenhossék employs Boveri's term "Centrosom" as a unit, designating by it a group of numerous very minute corpuscles held together by a "Zwischenmasse." He denies to each granule or corpuscle in the group the individual value which Heidenhain gives to each of his "Centralkörper." Von Lenhossék says ('95[a], p. 360): "Ich vermag die Beobachtungen Heidenhain's für mein Object vollauf zu bestätigen und möchte auf diese Zwischensubstanz besonderes Gewicht legen. Denn in ihrer Gegenwart erblicke ich dasjenige, wodurch das ganze Gebilde, mag es in seinem Innern noch so viele Körnchen beherbergen, doch immer in seiner Eigenschaft als *einheitlicher Körper* gewährleistet ist." And again (p. 361): "Somit könnte ich mich bei voller Anerkennung der Tragweite der Untersuchungen Flemming's und M. Heidenhain's der von diesen Forschern eingeführten Terminologie nicht anschliessen, bei der nicht das van Beneden-Boveri'sche Centrosom, sondern die darein eingebetteten *Körnchen* als Centralkörper benannt werden."

In none of the cells which I have studied do I find any such large number of minute granules held together by a "Zwischenmasse" as von Lenhossék describes. Often I have seen two, three, or four corpuscles in the centre of the sphere, but again, and more often, only one. This condition agrees better with the Microcenter and Centralkörper which Heidenhain describes.

The single central corpuscle, if only one is present, or the group of such corpuscles, if three or four are present, I call the centrosome. For the surrounding clear zone, the "Centralscheibe," described by von Lenhossék, it is best perhaps to employ his term "sphere," although the use of the term sphere for this structure is here open to the same objection as that which von Lenhossék urges: viz. it leaves out of account the coarsely granular zone, which he proposes to call the "Perisphere" or "Plasmasphere."

Dehler ('95) soon confirmed von Lenhossék's discovery. He found a similar condition in the sympathetic ganglion cells of the same animal, the frog.

Radiations from the region of the centrosome are neither described nor figured in the accounts given by either von Lenhossék or Dehler. The details of the "Centralkörpergruppe" figured by Dehler are much more nearly in agreement with the centrosome which I have described in this paper than is the centrosome of von Lenhossék. Dehler's "Centralkörpergruppe" agrees in its details with Heidenhain's Microcenter

rather than with von Lenhossék's "Centrosom," although Dehler does not admit for each corpuscle the individuality and value which Heidenhain attaches to each Centralkörper. Rather, he would limit such a value to the entire "Centralkörpergruppe," thereby showing himself in agreement with von Lenhossék.

A word may here be said upon the methods employed by von Lenhossék and Dehler. Both attach great importance to the use of corrosive sublimate followed by iron hæmatoxylin for the demonstration of the centrosome, and both state that treatment with osmic acid gives unsatisfactory results. Dehler (p. 725) says: "Ich halte für das Studium des *feineren Baues* der Nervenzelle die Fixierung mit Osmiumsäurelösung und ihren Combinationen für weniger brauchbar, da sich die feineren Gebilde diffus, verschwommen, eventuell schichtweise ungleich färben." In regard to the use of osmic acid, my experience has been very different, since I obtained my last and clearest preparations from material prepared by vom Rath's ('93, p. 102) osmic mixture. After reading von Lenhossék's paper, I took pains to confirm my results by his method, — the use of corrosive sublimate followed by iron hæmatoxylin, — and the results, although offering an excellent confirmation of the preparations made with the vom Rath mixture, were in no respect better.

A short time before the publication of Dehler's paper, Buehler ('95) described "Centralkörper," in the sense of Heidenhain's term, found in cells from the cortex of the forebrain of the lizard, and also in nerve cells of the human brain. He says (p. 230, Separate, pp. 32, 33): "Demgemäss bin ich vollauf berechtigt zu erklären: *Die Centralkörper, wie sie Heidenhain in Leukocyten von Salamander und Kaninchen und in Riesenzellen des letzteren Tieres beschreibt*, dieselben Centralkörper, die auch Flemming vor ihm an ruhenden Leukocyten und Gewebszellen fand, die eine Reihe anderer Forscher in verschiedenen Zellformen in Ruhe, unter anderm ich selbst an den Samenzellen des Krötenhodens, gefunden haben, *diese Centralkörper finden sich auch in den Ganglienzellen der Vorderhirnrinde der erwachsenen geschlechtsreifen Eidechse.*"

Buehler also found radiations arising from the Centralkörper, or from the group of Centralkörper. This is the only instance, save in that of my previous paper (Lewis, '96), in which this significant feature has been described or figured. Buehler says (p. 227, Separate, p. 19): "Nicht selten finden wir Linien im Protoplasma, die annähernd radiär verlaufend von den Kügelchen aus nach der Peripherie der Zelle hinstreben."

Schaffer ('96) announces a similar discovery for another vertebrate

type. He finds centrosomes in the "Schädelganglien von Petromyzon Planeri."

The first announcement of the presence of a centrosome in the nerve cells of an invertebrate was made by McClure ('96) for the unipolar ganglion cells of Helix. He gives no figures, but the brief description shows a condition corresponding in its principal features to the centrosome and sphere described by von Lenhossék.

When only von Lenhossék's and Dehler's papers had come into my hands, I gave my first account of the giant nerve cells of Clymene (Lewis, '96).* Further study has confirmed most of the statements made in that paper, and an opportunity for wider comparison with the statements of other writers strengthens the opinion then expressed, that this centrosome and sphere would be found to be of much more general occurrence in nerve cells than had hitherto been supposed.

Through the kindness of Mr. Hamaker of the Zoölogical Department of Harvard University, who has permitted me to see his preparations of the nervous system of Nereis, I am satisfied that he has demonstrated centrosomes in the giant cells of that annelid.

(2) *Function.*

Several facts suggest that this structure is a centre of mechanical activity in the cell: —

1) The concentric arrangement of the protoplasm around the centrosome.

2) The radiations which extend from the centrosome.

3) The strongly eccentric position of the nucleus.

4) The frequent flattening of the nuclear membrane on the side turned toward the centrosome.

5) The band of denser protoplasm partially surrounding the sphere, but interrupted by the nucleus.

I have no evidence that these cells undergo division, and it is pretty generally stated by authorities on nerve structures that ganglion cells after an early embryonic period never divide. Although I have sectioned and studied dozens of worms, I have never found the least indication of cell division in any cells of the nervous system of the adult.

As before stated, each of these giant cells has a single cell process.

* [Although a copy of Buehler's paper had, through the courtesy of the author, reached me on Dec. 30, 1895, by some mischance it was overlooked, and did not come to the attention of Miss Lewis until her paper ('96) on "Centrosome and Sphere," etc. had been published. — E. L. MARK.]

The place of emergence of this process is not constant, but usually it seems to arise from the cell at a point between the centrosome and the nucleus. Figures 33, 33ª, 34 (Plate **5**) show the relation of cell-process to nucleus and centrosome in several cases.

The most important facts regarding the giant cells of the nervous system of these two worms may be summarized as follows: —

1) The giant cells give rise to Leydig's fibres.

2) They are present in very limited numbers throughout the nerve cord; they show no regularity of arrangement, and no constancy in number in the different segments.

3) They are all characterized by the possession of a nucleus, eccentric in position, and by the presence of a centrosome and sphere.

III. PERIPHERAL NERVOUS SYSTEM.

1. Introduction.

This part of the paper, dealing with the peripheral nervous system of the two Maldanidæ, Axiothea torquata and Clymene producta, was presented in substance before the American Morphological Society in December, 1896, and was afterwards summarized in Science, Vol. V. No. 115, p. 428.

Under the term "epidermal sense organs" I include certain organs found abundantly on all the segments of the body in both worms mentioned. Similar organs have been referred to in the case of the *head* and *proboscis* of certain marine annelids. One writer only, so far as I know, has mentioned such organs as being found on other parts of the body of any marine annelid. Eisig ('87, p. 547) has described similar organs from both the abdominal and thoracic segments of two of the Capitellidæ. No one has given any account of the plan of distribution of such organs, nor has their structure, as determined by the newer nerve methods, been described. I have examined the removed cuticula of four marine worms, representing three different families, and have found evidences of such organs in all parts of the integument of the worm; I conclude, therefore, that, if not universally present on the body of polychætes, they are at least very common in marine worms.

2. Methods Employed.

In Clymene and Axiothea, three principal methods of study were employed, the results obtained by one being confirmed and extended by the others. They were as follows: (1) maceration, by which the cuticula

was removed entire for surface study; (2) sections, prepared either from material fixed in corrosive sublimate and stained with hæmatoxylin, or such as had been fixed in vom Rath's picro-osmic-acetic-platinic chloride mixture; (3) methylen blue employed upon living tissue, which was afterwards fixed, hardened, and sectioned.

For removing the cuticula the following procedure was found most successful. The worm was first narcotized in a mixture containing 95 volumes of sea water and 5 volumes of 95% alcohol, and then placed for a time, varying from 24 to 48 hours, in a 10% solution of sodium chloride. It was next removed to fresh water, and an incision through the cuticula of the back made with the points of scissors. The incision was carried along the whole dorsal line of the animal. A little shaking in the water with the forceps, aided with the scalpel, is sufficient to remove the cuticula entire, although it is usually most convenient first to cut the worms into pieces. The portions of cuticula thus obtained were floated upon slides; part of the water was drained off, and the rest allowed to evaporate. It is possible in this way to make permanent preparations of the cuticula. If the worm has been left in the salt solution a sufficient time, all the epidermal cells will remain behind, and a preparation of perfectly clean cuticula will be obtained. From such preparations the distribution of the sense organs could be determined easily by the use of low powers of the microscope. Preparations of this kind were also valuable for determining the presence and number of the canals serving for the passage of sensory hairs, and for comparing the sizes of the different sense organs.

Another method of maceration which was found fairly successful, although in general inferior to the one just described, was the use of a 1% solution of potassic bichromate. After the worm had been left in this solution for several weeks, fairly large pieces of cuticula could be removed.

For the study of the finer structure of the sense organs, material prepared by the vom Rath method gave the most satisfactory results. By this method, sense hairs and cuticula, as well as the cells of the epidermis, were well preserved. It was found to be important to use great care in narcotizing the worms before placing them in the killing fluid, for otherwise the sense organs are contracted so as to be worthless.

Sections from material fixed in mercuric chloride and stained with hæmatoxylin showed plainly the presence of the organs and the peculiar condition of the cuticula above them; but the sense hairs were matted together, and in other respects the preparations were inferior to those prepared by the vom Rath method.

After the presence of these sense organs had been established and their finer structure studied by the methods described, I was able to apply successfully the methylen blue method to living material. The results thus obtained confirmed what had already been found, and gave some additional facts. The methylen blue was used in the following manner. The worms were first narcotized with chloroform and then a 1.5% solution of methylen blue in normal salt solution (0.66%) was injected into the body cavity, the injection often being made in two or more regions of the body. The worm was then put back into sea water. After a few minutes a second injection, similar to the first, was made, and the worm was again returned to the sea water. In the course of five or six hours the sense organs gave evidence of being stained. The worms were then placed in Bethe's ('96) mixture for invertebrates to fix the blue, afterwards dehydrated in alcohol, embedded in paraffine, and cut into sections as thick as practicable. Grübler's B. X. brand of methylen blue proved to be the most satisfactory. The results obtained by the methylen blue method will be considered later. I will pass now to the consideration of the sense organs.

3. Distribution of Sense Organs.

Both Axiothea torquata and Clymene producta have two well marked body regions, separated from each other by a sort of collar. These two regions I call thoracic and abdominal, using the terms employed by Eisig for the body parts of the Capitellidæ. The distribution of the epidermal sense organs over these regions is as follows. (See Plate **8**, Fig. 68.)

a. *In the Thoracic Region.*

The thoracic region consists in both genera of the first four segments. The posterior part of the buccal segment and the anterior portion of the one next following show a peculiar mosaic arrangement of the surface not found in any other portion of the worm. The mosaic-like patches are bounded by irregular linear grooves, and their surface is slightly raised. Upon these patches the sense organs (represented in Fig. 68 by dots) are found in abundance. In the grooves both sense organs and gland pores are wanting. It is evident that the sense organs, being situated upon these patches, are more exposed to contact from without than they would be if they were located in the grooves.

The proboscis is longitudinally ribbed, and the sense organs upon it show a corresponding arrangement, for they are found upon the longitudinal ridges, not in the grooves.

Upon the crest of the head (see Lewis, '97, Plate **1**, Figs. 2 and 3) there are many sense organs of large size.

With the first setigerous segment (Plate **8**, Fig. 68) begins a well marked grouping of the sense organs, some 15 to 20 in number, posterior to and in the immediate vicinity of the ventral setæ. A similar grouping is shown in all the thoracic segments. At the anterior and posterior margins of the thoracic segments the sense organs are more numerous, forming ill defined circular bands, but in other portions of the segment they are rather uniformly distributed, though without any distinct plan.

b. *In the Abdominal Region.*

It is more difficult to study the sense organs in the abdominal region, on account of the extreme thinness of the cuticula; but by careful examination with high powers such organs can always be found. Toward the posterior extremity of the worm the cuticula again becomes thicker and the sense organs more prominent. The last segment of the tail ends in a funnel-shaped expansion within which numerous sense organs are found. Such a conspicuous grouping of sense organs as is seen in the thorax cannot be made out in the abdomen, although it seems evident that here too there is a slight concentration of organs around the setæ and at the anterior and posterior margins of the segments.

As a rule the sense organs vary in size directly with the thickness of the cuticula.

They resemble somewhat those described by various writers for Lumbricus, but differ from them in the following respects, at least: (1) no sheath cells are present; (2) gland pores are not absent from the region immediately surrounding them; and (3) the number of cells composing an organ is much smaller than in the case of Lumbricus.

4. Structure of Epidermis.

The epidermis of both these annelids shows three types of cells: (1) ordinary epidermal cells, (2) gland cells, which are very numerous in certain regions, and (3) the cells of the sense organs.

a. *Ordinary Epidermal Cells.*

The ordinary epidermal cells (Plate **7**, Figs. 63–65), the thread cells of certain writers, show great variation of form in different regions of the body. They are somewhat cylindrical, have an elongated nucleus and possess usually two or more thread-like roots, as long as the rest of the cell. The nucleus lies immediately above the point where the roots

unite. In certain regions of the worm these cells are greatly elongated and very slender. Those shown in Figures 63–65 were stained in methylen blue.

b. *Gland Cells.*

The gland cells (Plate **3**, Figs. 17–20) are especially numerous in the fifth to the ninth segment of the body inclusive. They also show great variation in size and shape. The gland pore, situated at the free end of the cell, is readily seen in sections (Fig. 18) or in mounted specimens of removed cuticula (Fig. 19).

c. *Sense Organs.*

I propose to describe the sense organs somewhat minutely. Their appearance will be considered, first, in the removed cuticula; secondly, in sections prepared by ordinary methods and by the vom Rath fixative; and, thirdly, in methylen blue preparations. Conclusions as to their finer structure and their probable functions, and a historical review follow.

(1) *Cuticula.*

A study of the removed cuticula shows that it is everywhere made up of two systems of fibres, which cross each other at an angle of about 90° (Plate **3**, Fig. 19). Both systems are continuous, though faint, over each sense organ. The position of the sense organs is marked by a differentiation of the cuticula, which is nearly circular in outline. The cuticula of these areas is much reduced in thickness, but seems to be more highly refractive than the surrounding cuticula. No gland pores are present in these circular areas, but study with high powers reveals a number of very minute openings, — the canals through which the sense hairs pass. The number of these canals varies. I have counted as many as nine or ten in a single area. The diameter of the areas varies from 8μ to 16μ, and may even exceed this size. Frequently one or more cells from a sense organ are left hanging to the dried cuticula; in such cases the slender peripheral cell-process running up to the pore canal may be readily made out. Figure 19 (Plate **3**) is a camera drawing showing the appearance of the surface of a portion of the cuticula including one of these areas, made from the removed cuticula of Axiothea torquata. A section perpendicular to the surface of the body through one of the sense organs, from material fixed with the vom Rath fluid, is shown in each of the Figures 17 and 18 at the top of Plate **3**. From these, as well as from Figures 35–46 (Plate **6**) and 48–52 (Plate **7**), it is to be seen that im-

mediately over the sense organs the cuticula is of less than half its usual thickness. It is deeply concave on the inner surface, and shows on the exterior a slight convexity, which is usually surrounded by a shallow circular depression.

(2) *Sensory Cells.*

After having given considerable attention to the matter, I have come to the conclusion that the sense organs of Clymene producta and Axiothea torquata are invariably composed of a number of spindle-shaped cells, — that there are no single or isolated sensory cells. The best general view of the sensory organ is obtained from sections perpendicular to the surface of the worm, made from material fixed in vom Rath's mixture, such as is shown, in part, in Figures 17 and 18 (Plate **3**). From sections of this kind it is to be seen that each sensory organ is composed of a small number — usually a dozen or less — of closely grouped spindle-shaped cells traceable through nearly the whole thickness of the epidermis. Near their basal ends these cells may take different directions, so that the bundle becomes dissolved before reaching the basement membrane. In such sections the exact shape of that portion of the sensory cell which lies next the cuticula could not be fully made out; but the grouping of the cells, the spindle-shaped body of each cell, and the sensory hairs passing through the cuticula, are all plainly visible. By no other method were the sense hairs so satisfactorily shown. Occasionally (Fig. 17) the cuticula is slightly pulled away from the cells of the sense organs, and in such cases the sensory hairs may remain in the cuticula, projecting beyond its free surface. Although the exact form of the peripheral portion of each cell could not be satisfactorily made out from such sections, yet enough could be seen to show that the cells are elongated and spindle-shaped, tapering both above and below the nucleus, which is oval, rather large, and contains a single nucleolus. Near the cuticula, the cells are closely pressed together, their peripheral ends being evidently very slender. In most cells the nucleus is situated at about half the height of the epidermis, but in some it may be near the cuticula and in others sunk to the base of the epidermis. This difference in the position of the nucleus is often shown in the different cells making up a single organ.

(3) *Results of Methylen Blue Method.*

The application of the methylen blue method confirmed the results obtained by other methods, and gave much additional information concerning the individual cells of the sense organs and their relation to nerve

fibres. The results of the injection of methylen blue were studied both in the living tissue and in sections prepared after treatment by the ammonium-molybdate method of Bethe.

To study the living tissue of worms that had been injected with methylen blue, portions of the body wall in the head region were cut out, placed upon a slide, and observed under a cover glass after adding a few drops of sea water. The positions of the sense organs could be readily determined, but the thickness of the cuticula prevented any satisfactory conclusions as to the shape and number of the cells stained. Owing to the opacity of such preparations, the central processes of the cells could usually be followed only a very short distance. The sensory hairs often took the blue stain.

Two such hairs from an organ which had taken the blue stain were seen in one instance to move for a considerable time, and their position with reference to each other was seen to change. This was so strange a phenomenon, that I asked a friend to examine the living preparation at the time, and he confirmed my conclusion. So far as I know, however, there exists no observation to show that sensory hairs are ever capable of independent motion.

The results obtained from *sections* of the methylen blue material were far more complete and instructive. A comparative study of many sections was necessary, however, to give a satisfactory idea of a sense organ.

Often but one of the cells of the group making up an organ took the methylen blue stain, and from such preparations the hasty conclusion might have been drawn that only single isolated sense cells were under examination. A careful examination of the cuticula, however, almost always showed the presence of a concave inner edge and the doubly curved outer edge characteristic of the cuticula of a sense organ composed of several cells. In the very few cases in which this condition of the cuticula did not appear, there seemed reason to believe its absence due to a slight obliquity of the section.

Sometimes the blue of the stain was concentrated upon the concave inner surface of the cuticula covering the sense organ. In this way the presence of several separate organs could be recognized, all other portions of the cuticula remaining uncolored.

In other instances, all or nearly all the cells of an organ would be stained; in such cases little could be made out from the thick section, owing to the closeness of the opaque cells.

The most satisfactory conditions were perhaps those (Plate **6**, Figs. 37,

38, 42–44, 47) in which only two or three of the cells of an organ had taken the stain, and that not too intensely, thus allowing the two poles of the cells to be traced, — the peripheral pole through, or partly through, the cuticula, the central pole for some distance toward the central nervous system.

(4) *Conclusions as to Finer Structure of Sense Organs.*

From the study of a large number of preparations made by the different methods described, I have reached these conclusions.

(1) That epidermal sense organs composed of a number of sense cells are present in all parts of the integument of the Maldanids, Clymene producta and Axiothea torquata, and that in certain regions of the worm these sense organs show a definite arrangement into groups and zones.

(2) That the cells of these sense organs are elongated, spindle-shaped cells, — bipolar nerve cells of the type described by Retzius ('92, '92[a], and '95) for the isolated sense cells of Nereis.

(3) That these bipolar cells differ much in the distance of the nucleus from the cuticula.

(4) That there are a considerable number of such bipolar cells in each sense organ, although in many cases only one cell takes the stain. In almost all cases in which only one cell is stained, the peculiar contours of the cuticula in sections give satisfactory evidence of the presence of a multicellular sense organ.

(5) That each of these cells possesses at its exterior end a sense hair. These hairs I believe capable of retraction below the cuticula, although I cannot offer conclusive evidence upon this point.

(6) That the deep portion of each of these cells is much more slender than the peripheral portion, and turns at an angle beneath the epidermis toward the central nervous system. But not all the processes from the central ends of the cells of an organ pass in the same direction. I have seen them in some instances separate when near the base of the epidermis, and diverge until they took opposite directions (Plate **6**, Fig. 42), in which they could be followed for some distance. This would seem to indicate that nerve fibres from the same sense organ may enter the central nervous system from opposite sides. The exact manner in which the nerve fibres from these sensory cells enter the central nervous system I have not been able to observe.

(7) That the direction of the cells of the sense organs in reference to the cuticula varies considerably. As a rule, the long axis of the cell body is nearly perpendicular to the cuticula, and to the circular muscles.

But many cells show an oblique direction of the long axis with reference to the cuticula (see Plate **6**, Fig. 38, and Plate **7**, Fig. 51). It is evident that ordinary methods of staining would be very unsatisfactory for the discovery of these oblique cells.

(8) That in many respects the sensory cells of these epidermal sense organs show a remarkable resemblance to those described by Retzius ('92, '92[a], and '95) for Nereis. The chief point of difference is, that Retzius found *isolated* sensory cells in the case of Nereis, whereas in the case of these Maldanids the sense cells are grouped into definite sense organs. Is it not possible that the conditions seen and figured by Retzius may be accounted for without concluding, as Retzius has done, that sensory cells exist in the epidermis of Nereis as isolated single-cell organs? Is it not possible that Retzius, through failure to control his observations by sections made after the more ordinary methods of treatment, overlooked the fact that such apparently isolated cells really belonged to a multicellular sense organ?

(9) That the epidermal sense organs here described can be observed in the living worm into which methylen blue has been injected, the worm being placed in a shallow dish of sea water, and examined even under a comparatively low power of the microscope.

(5) *Function of Sense Organs.*

Regarding the function of these sense organs, I have little to suggest save of a negative character.

The fact that they are found at all parts of the surface of a worm inhabiting a tube would seem to be against the supposition that they are organs of taste. In such a worm organs of taste would be of little service except at the anterior region of the body. The same argument may be made against the supposition that they are organs of smell.

Certain facts seem to favor the supposition that they are tactile organs. That the worm is sensitive, even in the posterior region of the body, is shown by pinching or pricking the tail as it projects from the sand tube which it inhabits. Again, we find the organs especially numerous upon exposed or elevated parts of the body.

Spengel, who found similar organs, but only in the mouth region of certain marine annelids, held them to be organs of taste on account of their position in the mouth; but this reasoning could not hold for organs found in other regions of the body. It is not impossible, of course, that in the region of the mouth such organs function as organs of taste, and in other parts of the body as tactile organs.

(6) *Historical Review.*

In going over the literature concerning epidermal sense organs in annelids, I have found that only a very few of the writers upon polychætes have made any reference to such organs as those which I have described, and in no case have I found any account of the finer structure of those organs which agrees with the results that I have obtained. I shall refer in chronological order to the writings of such authors as seem to have had under investigation organs similar to those of Axiothea and Clymene.

Claparède ('68), in his account of Nepthys scolopendroides, describes in a general way, under the head "papilles de la trompe," structures which may possibly be the same as the epidermal sense organs of these Maldanidæ. He says (p. 487): "Les papilles qui bordent l'ouverture de la trompe portent des terminaisons nerveuses, très-remarquables, qui font défaut aux cercles de papilles plus extréieurs. Ces papilles sont triangulaires et de deux espèces: les unes larges et les autres minces. Ces deux formes alternent régulièrement l'une avec l'autre. La première seule porte les organes en question, sous la forme d'une véritable forêt de longues soies délicates, ondulées, fort ténues. Elle sont situées à la base de la papille. Quelques faisceaux de nombreuses soies semblables se voient encore vers le milieu de la papille. En revanche, le sommet de celle-ci en est dépourvu et ne présente que quelques petites éminences striées à peine appréciables, rappelant les organes tactiles des palpes des Néréides. Les soies que je viens de décrire ne sont reconnaissables qu'à l'aide de forts objectifs. La question de savoir si ce sont des éléments nerveux est sans doute indécise. Le nerf de chaque papille s'épanouit en un pinceau dont les fibres viennent aboutir sous la cuticule fort amincie. Il est par suite facile de supposer une continuité entre les fibres et les soies ondulées de la surface. Toutefois, je reconnais que cette continuité ne peut guère être un fait d'observation."

Spengel ('81), describing the folds of the proboscis in certain polychæte annelids, says (p. 21): "Kaum minder stark entwickelt als bei Lumbriconereis sind solche Falten bei Arabella und namentlich bei Halla. Bei letzterer Gattung sind sie der Sitz wohl ausgebildeter 'becherförmiger Organe,' die empfehlenswerthe Objecte zum genauren Studium dieser so weit verbreiteten Form des Sinnesepithels sein dürften. Die Anordnung derselben erhellt aus Fig. 32, was ich von ihrer Structur erkannt habe, aus Fig. 33. Dieselben sind dadurch ausgezeichnet, dass sie von einer dicken, hellen, aber von feinen Poren durchbrochenen Cuticula bedeckt sind; dass diese Poren zum Durchtritte von Sinneshaaren dienen werden,

ist in hohem Grade wahrscheinlich, liess sich indessen an den conservirten Thieren nicht mehr nachweisen. Die Sinnesorgane selbst sind aus hellen, mit einem länglichen Kerne etwas unterhalb ihrer Mitte versehenen Cylinderzellen gebildet, die einen etwa kugligen Körper darstellen. Die Lage lässt in denselben Geschmachsorgane vermuthen. Auch bei Lumbriconereis sind ähnliche Sinnesorgane vorhanden, doch minder scharf differenzirt." It is probable that the structures which Spengel describes are similar to those described in the present paper.

Eisig ('87) describes in his monograph of the Capitellidæ, under the name "becherförmige Organe," structures which are undoubtedly equivalent to the sense organs of the Maldanids here described. He states that they are found on the head, proboscis, and thorax of all the Capitellidæ, and in case of two species of the family, besides being found in these regions, they are also present on the abdomen. No other author has, so far as I know, mentioned such organs in case of polychætes for any region except the proboscis. Regarding their distribution, Eisig says (p. 547): "Im Gegensatze zu den streng metamerangeordneten Seitenorganen zeigen die becherförmigen Organe eine von der Körpersegmentirung durchaus unabhängige, also *diffuse Vertheilung*." And concerning their structure he adds (p. 547): "Was die Structur der becherförmigen Organe betrifft, so sind auch bei ihnen als auffallendste Theile die auf den Hügelkuppen concentrirten, frei in das Medium hinausragenden Sinneshaare hervorzuheben. Diese Haare sind etwa 4μ lang, wenig zahlreich und überall gleich breit, also stäbchenförmig. So wie bei den Seitenorganen durchsetzen sie die die Hügel überziehende Cuticula, um in ein Bündel central gelegener, langgestreckter Sinneszellen überzugehen. In letzteren Sinneszellen erkennt man ohne Weiteres den Typus der Hautfadenzellen wieder."

In regard to the innervation of these "becherförmige Organe," Eisig gives the following (p. 548): "Meine bezüglichen Nachforschungen hatten denn auch keinerlei derartiges Resultat zur Folge, so dass wir wohl annehmen müssen, dass die Sinneszellen der Becherorgane ebenso wie die Fadenzellen der Haut von dem integumentalen Ganglienzellenplexus aus mit Nervenfibrillen versorgt werden."

It is evident that Eisig's results concerning the finer structure of these sense organs, i. e. concerning the form of the sensory cells and their innervation, have not much in common with the results which have been given in this paper. The fact that none of the finer methods of nerve technique were employed by the authors hitherto quoted probably accounts adequately for the points of difference between their conclusions and mine.

I come now to the consideration of the work which has been done upon the sense organs of polychæte annelids by means of the newer nerve methods. Retzius, working both with the Golgi method and with methylen blue in 1892, and confirming in 1895 his previous work, describes for the epidermis of Nereis certain *isolated* sensory cells, which in almost all the details of their structure and in their variations of form and position show a remarkable resemblance to the cells of the sense organs which I have described. I, however, have never found the cell body and nucleus sunk beneath the deep ends of the epidermal cells into the underlying tissue, as Retzius has. I find some of the nuclei of the sensory cells, it is true, at the most variable distances beneath the cuticula, even as deep as the base of the epidermis, where they may lie immediately above the circular muscles, either in an oblique position or parallel with those muscles, and it not infrequently happens that such a nucleus is situated at some distance from the vertical axis of the sense organ to which it belongs.

For this apparent difference of condition between Nereis and the Maldanidæ, there are three possible explanations: —

(1) The Maldanidæ studied by me may have had in the epidermis isolated sense cells which did not respond to the blue method and were consequently overlooked; and on the other hand Nereis may have contained sense organs similar to those of the Maldanidæ, which did not respond to the methods employed by Retzius.

(2) As Retzius used in his work only the Golgi and the methylen blue methods, neither of which are especially favorable for the preservation of the outline of the cuticula above the sense organs, he may have overlooked any such indication of multicellular sense organs. Moreover, since frequently only a single cell from such an organ stains with the blue, he may have seen only such conditions and have consequently interpreted them as isolated sense cells.

(3) The epidermis of Nereis may differ so much from that of these Maldanidæ as to have only isolated sense cells, while the Maldanidæ have sense cells grouped into sense organs. This third supposition, however, I think unwarranted. For although I have studied Nereis by means of the removed cuticula only, I am convinced from the examination of this cuticula that organs resembling those which I have described for the Maldanidæ are likewise present in their integument.*

* Since this part of the present paper was completed, the report of the meeting of the American Morphological Society for December, 1896, has been received

Before concluding this paper I would take the opportunity to express my sincere gratitude to the director of my studies, Professor E. L. Mark, for the assistance and advice which I have constantly received from him. And I would gratefully acknowledge my indebtedness to the officials of Radcliffe College for the encouragement which they have given me in my work, and to the Society of Collegiate Alumnæ for the aid I have received from them in the form of a fellowship.

IV. SUMMARY.

1. Confirmation of the view that Leydig's fibres in annelids are true nerve fibres; that the sheath of Leydig's fibre is comparable to the medullary sheath of nerve fibres in vertebrates; that the contents are comparable to the axis cylinder; and hence, as has been pointed out by Friedlaender, that the line which has been drawn between the nerve fibres of vertebrates and invertebrates cannot be maintained.

2. Confirmation for Maldanidæ of the theory that Leydig's fibres do not function in any way as an organ of support. They are neither homologous nor analogous to the chorda dorsalis of vertebrates.

3. Confirmation of the view that Leydig's fibres result from the union of the direct processes of giant ganglion cells. In Axiothea torquata and Clymene producta these cells appear in the sub-œsophageal ganglion, and are found scattered along the lateral and ventral portions of the

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(Science, Vol. V. No. 115, March 12, pp. 423–436). This report contains (pp. 427, 428) an abstract of a paper by Miss F. E. Langdon on "The Peripheral Nervous System of Nereis virens." The writer says: "The spindle-shaped sensory cells described by Retzius as isolated are really grouped into semi-organs, which have a definite distribution over the body. Each organ consists of a fusiform group of cells, whose bodies lie below the epidermis or in its base. The cuticular markings over the organs in the appendages of the body are like those over the sense organs of Lumbricus. Over the body itself each cuticular marking is concave on the exterior, and the very thick cuticula encloses beneath each marking an ovoid cavity, through which pass the outer ends of the sensory cells. Each sensory cell usually bears several sensory hairs, and these hairs cannot be retracted normally as supposed by Retzius."

There is one noteworthy difference between this description and that which I have given for the epidermal sense organs of the Maldanidæ. I have found *only a single sensory hair to each sense cell* in case of the Maldanidæ studied, whereas Miss Langdon has found that in the case of Nereis *each sensory cell usually bears several sensory hairs.* We are, however, in agreement as to the grouping of the sensory cells into multicellular organs.

nerve cord without indicating metamerism or symmetry. In the sub-œsophageal ganglion, however, they show an approach to symmetry.

4. Confirmation of the conclusion that the substance of Leydig's fibre is uniform throughout and does not represent a bundle of nerve fibrillæ; further, that the fibre does not show any indication of being a degenerate structure.

5. In their relation to ganglion cells, Leydig's fibres, if real nerve fibres, as maintained, stand in strong opposition to the neuron theory of Waldeyer, von Lenhossék, Edinger, and other writers. The relation of the process of one giant cell with that of another appears to be one of direct continuity, not simply of contiguity.

6. The cells which give rise to Leydig's fibres show peculiar structural conditions in the possession of a nucleus always eccentric in position and in the possession of other structures more central in position, the centrosome and sphere.

7. The presence of a centrosome in these ganglion cells argues for a function of the centrosome other than that of an organ for cell division.* No division of nerve cells has been observed in this worm, and, besides, cell division is generally believed never to occur in case of normal ganglion cells.

8. Although not an organ of cell division, the centrosome in these ganglion cells is evidently a centre of mechanical activity. This is indicated, (*a*) by the eccentric position of the nucleus; (*b*) by the flattening or indentation of the nuclear membrane, frequently observed on the side toward the centrosome; (*c*) by the concentric arrangement of the protoplasm around the centrosome; and (*d*) by the radiations which extend from the centrosome.

9. The presence of a centrosome in ganglionic cells is an argument in favor of its being a permanent cell organ.

10. In some of the giant cells two centrosomes and spheres are present, without there being any evidence of approaching nuclear division.

11. Parts of the peripheral nervous systems of the two Maldanidæ, Axiothea torquata and Clymene producta, terminate in multicellular

[* The identity of the centrosome here described with that which usually accompanies cell division is here assumed; but it has not been proved. An important problem for the future is to determine if a genetic connection can be established between this so called centrosome of nerve (and other) cells and the centrosome which exists during cell division. — E. L. MARK.]

sense organs occurring abundantly throughout the integument of both worms.

12. These sense organs show in certain regions of the body a definite plan of distribution, being collected into rows, groups, and zones.

13. Isolated sense cells are not present in the epidermis.

14. The individual cells which make up the sense organs are bipolar nerve cells, resembling the isolated sense cells which Retzius described for the epidermis of Nereis. The peripheral prolongation of each cell carries at its free end a single sensory hair; the prolongation of the deep end has not been completely followed out, but as far as traced is unbranched, and represents a nerve fibril.

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EXPLANATION OF PLATES.

All the figures in Plates **1–5**, and **8**, except Figures 19, 20, and 68, represent preparations from *Clymene producta*. Figures 19, 20, and 68, and all the Figures of Plates **6** and **7** represent preparations from *Axiothea torquata*.

The magnification of all the giant cells is 1000 diameters, and the method of preparation, unless otherwise stated, was by the vom Rath mixture, defined at page 232.

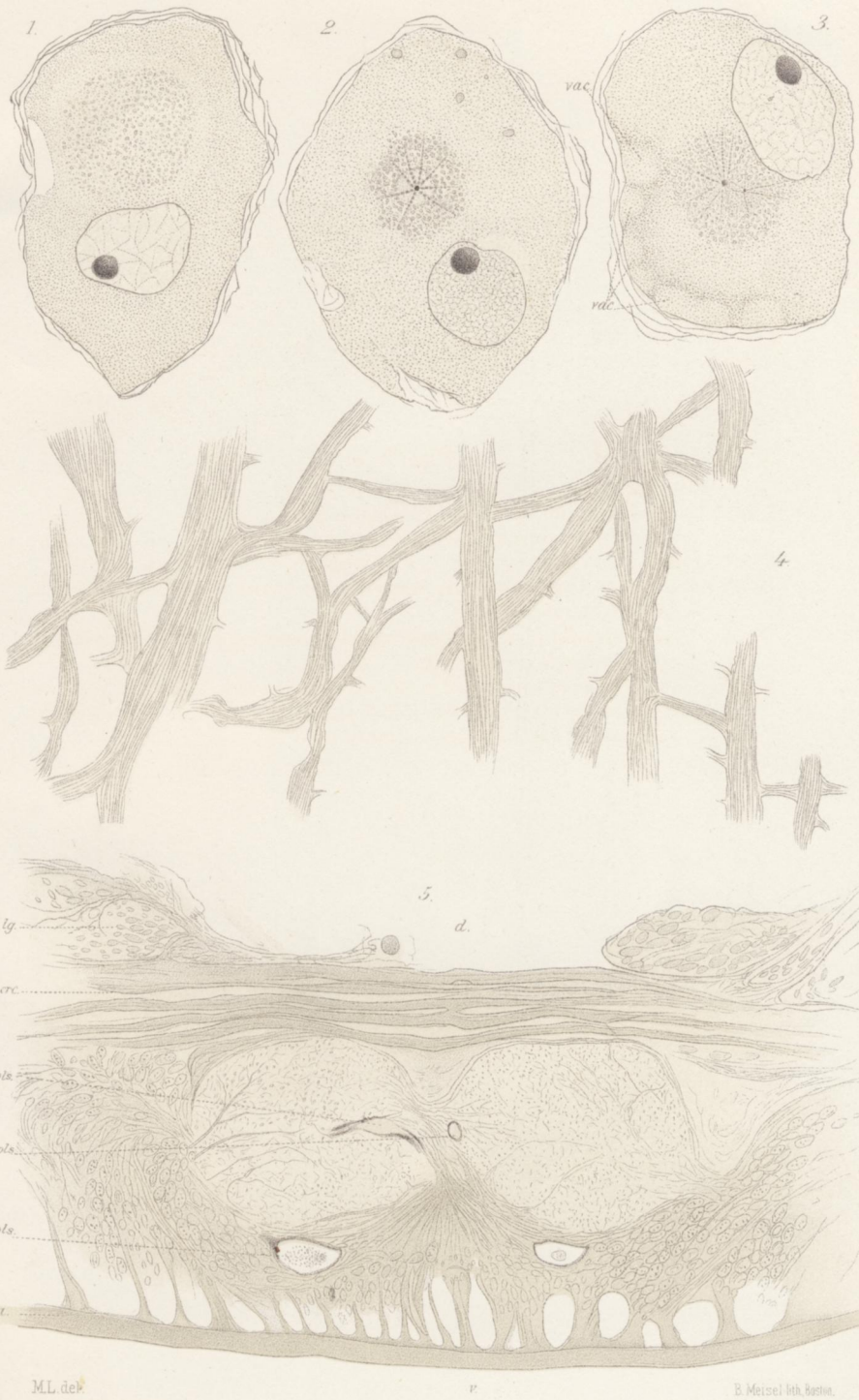
All figures were drawn with the aid of the Abbé camera lucida.

ABBREVIATIONS.

A.	Anterior.	*med.*	Medullary sheath.
can. gl.	Pore canal of gland.	*mu. crc.*	Circular muscles.
cd. n.	Nerve cord.	*mu. lg.*	Longitudinal muscles.
cl. cols.	Giant cell.	*o. sns.*	Sense organ.
cl. gl.	Gland cell.	*P.*	Posterior.
cl. h'drm.	Hypodermis cell.	*pli. pr'b.*	Folds of proboscis.
cl. n.	Nerve cell.	*po. gl.*	Gland pore.
cl. sns.	Sense cell.	*set.*	Seta.
c'sph.	Centrosphere.	*set. sns.*	Sense hair.
cta.	Cuticula.	*v.*	Ventral.
d.	Dorsal.	*vac.*	Vacuole.
fbr. cols.	Leydig's fibre.		

PLATE 1.

Figs. 1–3. Sections from three giant ganglion cells.
Fig. 1. Section showing relation of nucleus and sphere.
Fig. 2. Section showing centrosome and centrosphere with radiations.
Fig. 3. Section showing in addition flattening of nuclear membrane on the side toward the sphere.
Fig. 4. Tangential section of body wall to show branching and anastomosing of nerves beneath the hypodermis. × 300.
Fig. 5. Cross section of nerve cord in the region of the sub-œsophageal ganglion, showing the symmetrically placed giant cells and cross sections of the two forks of Leydig's fibre. (Drawn by K. Hyashi.)



M.L. del.

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B. Meisel lith. Boston.

PLATE 2.

Figs. 6, 7. Diagrams of the nerve cord in the ninth and tenth segments respectively, to show the distribution of the giant ganglion cells and their relation to Leydig's fibre. The two horizontal lines mark the position of the setæ. The two figures are to be imagined as united at β.

Figs. 8, 9. Sections of different giant ganglion cells from the region of the subœsophageal ganglion, showing in each cell two spheres, each with centrosome and radiations. The nucleus was not cut in either case.

Fig. 10. Section of giant ganglion cell, showing an outpocketing of the cell in which the nucleus lies.

Figs. 11, 12. Sections of different ganglion cells, showing centrosome and radiations.

Figs. 13, 14. Two successive sections of the same cell, showing nucleus and two centrospheres.

N. B. By an error in transferring, Figure 14 is upside down.

Fig. 15. Cross section of Leydig's fibre. × 1000.

Fig. 16. Longitudinal section of Leydig's fibre. × 1000.

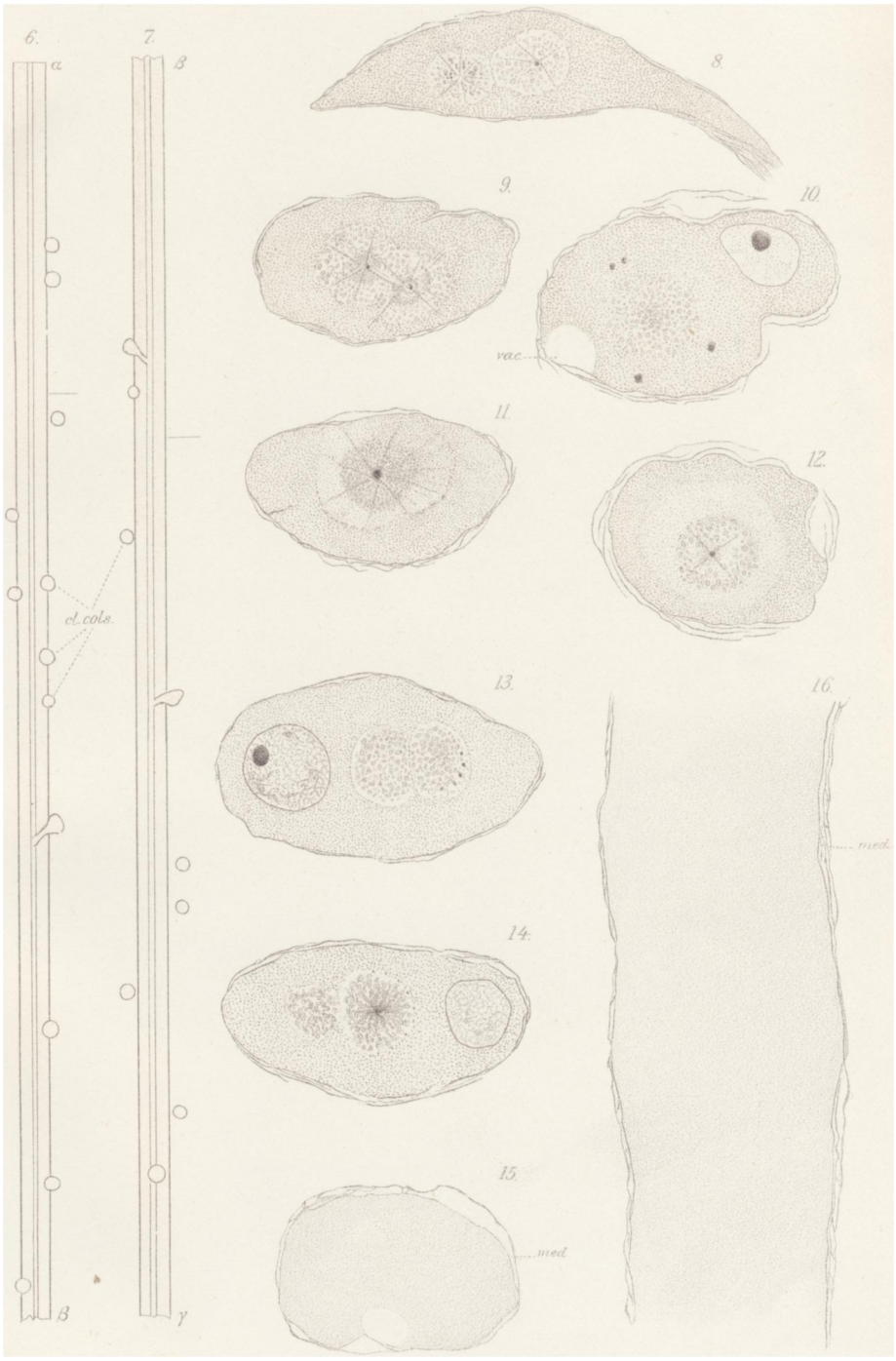

6.
α
B
7.
β
γ
8.
9.
10.
vac.
11.
12.
cl. cols.
13.
14.
15.
med.
16.
med.

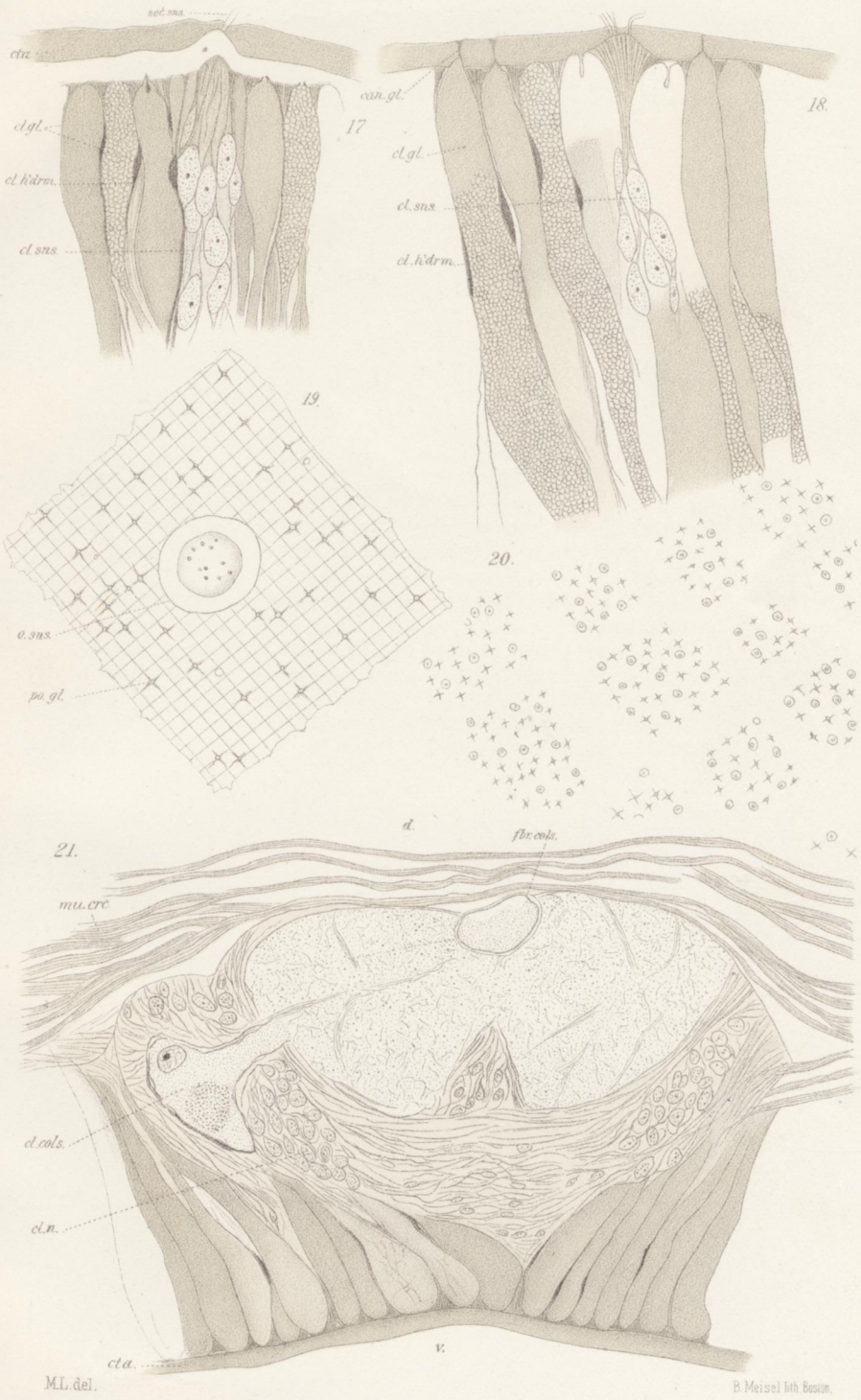
PLATE 3.

Figs. 17, 18. Sections of hypodermis, each passing through a single sense organ, and showing sensory hairs. Vom Rath preparations. × 640.

Fig. 19. Piece of removed cuticula, highly magnified, and showing the area over a sense organ. The minute circles within the circular area mark the positions of pore canals accommodating the sensory hairs. The small circles outside the area represent gland pores. × 1000.

Fig. 20. Piece of removed cuticula from the first segment, showing the mosaic patches upon which the sense organs are found. These are indicated by concentric circles, some of the gland pores by a small circle and cross. × 120.

Fig. 21. Cross section of nerve cord and body wall, to show topographical relations. Vom Rath preparation. × 640.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fbr.cols.
mu.crc.
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M.L. del.
B. Meisel lith. Boston.

PLATE 4.

Figs. 22, 23. Successive longitudinal sections of nerve cord, showing relation of giant ganglion cell to Leydig's fibre. Vom Rath preparation. × 750.

Fig. 24. Section of giant cell, showing centrosome composed of several corpuscles.

Fig. 25. Section of giant cell, showing eccentric position of the nucleus and the band of denser protoplasm surrounding the sphere.

Fig. 26. Section of giant cell, showing centrosome with radiations and pole of the cell. The protoplasm is slightly shrunken away from the sheath of the cell on one side. Iron hæmatoxylin preparation.

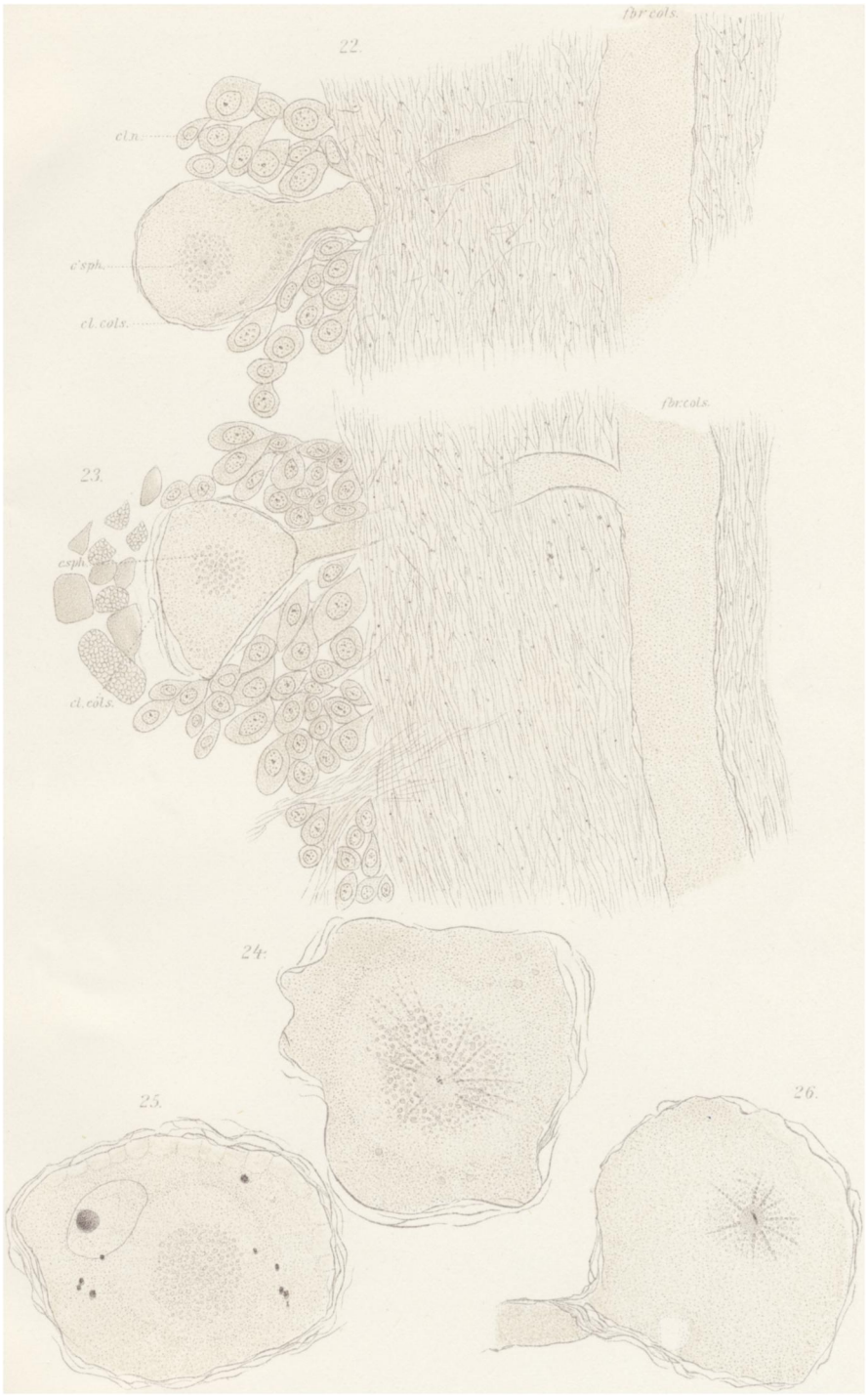

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PLATE 5.

Figs. 27–30. Four successive sections through one cell.

Fig. 27. Section through nucleus, showing two nucleoli.

Fig. 28. Section through nucleus and outer zone of sphere.

Fig. 29. Section through sphere and centrosome. Both Figures 28 and 29 show the band of denser protoplasm partially surrounding the sphere.

Fig. 30. Section through sphere and centrosome, showing a central corpuscle, probably not the one shown in Figure 29.

Fig. 31. Section of giant cell, showing the outpocketing of the cell in which the nucleus is contained.

Fig. 31ª. Section of cell from the region of the sub-œsophageal ganglion, showing nucleus and two spheres.

Fig. 32. Section immediately following the one shown in Figure 31. Radiations extend far out through the protoplasm of the cell.

Figs. 33, 33ª, 34. Sections through different giant cells, to show relation of the sphere and nucleus to the cell process.

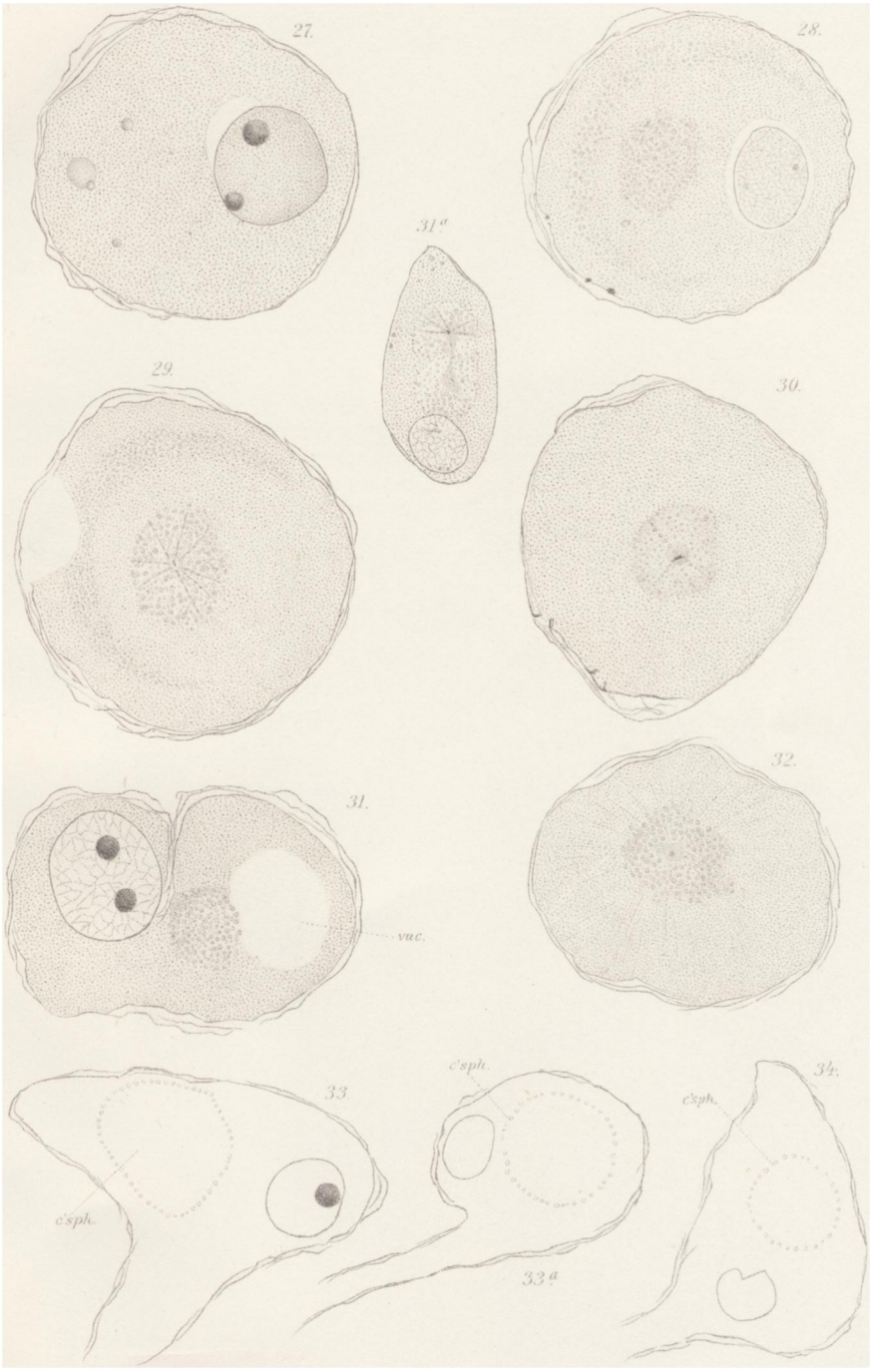

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PLATE 6.

All the figures shown in Plate 6 represent sections through sense organs from *Axiothea torquata* stained with methylen blue. All are drawn to a magnification of 750 diameters, with the exception of Figures 37 and 46, which were drawn under a $\frac{1}{18}$ (Zeiss) homogenous immersion lens.

Fig. 37. Section showing the peripheral portion of the cells drawn in Figure 38, more highly magnified.

Fig. 44. Section through a sense organ on the ventral side of the worm, showing one cell belonging to the sense organ, and four belonging to the central nervous system (compare Plate 3, Fig. 21, *cl. n.*). In two cells the nucleus was clearly distinguishable; in two it was not. The single continuous line farthest from the cuticula indicates the boundary of the fibrous portion of the ventral nerve cord.

Fig. 47. The line farthest from the cuticula indicates the boundary of circular muscles.

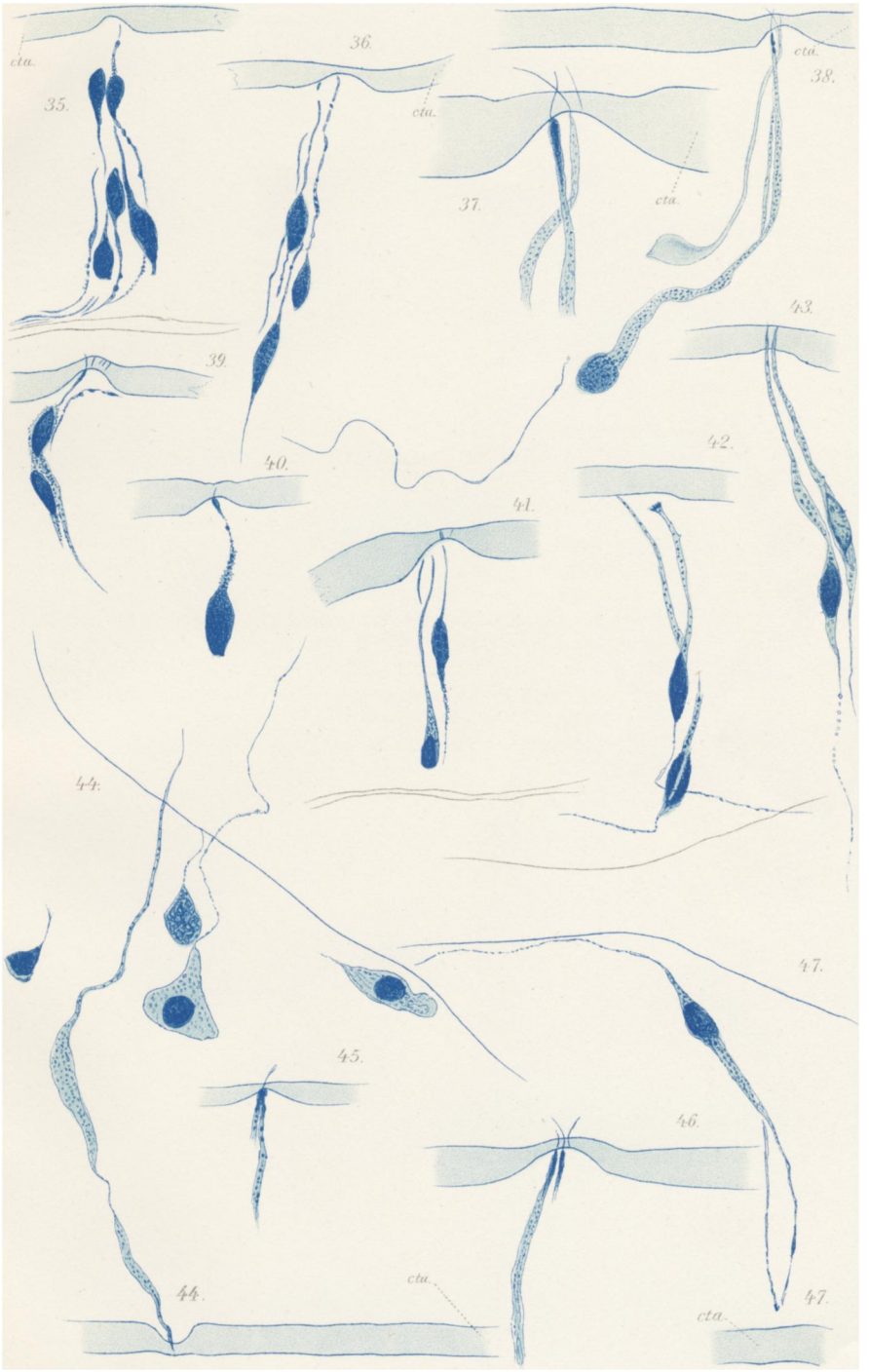

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PLATE 7.

Figs. 48–52. Sections through sense organs of *A. torquata*, showing sensory cells stained with methylen blue. × 750.

Figs. 53–62. Cells from the central nervous system of *A. torquata*, stained with methylen blue. × 750.

Figs. 63–65. Ordinary hypodermis cells from *A. torquata*, stained with methylen blue. × 750.

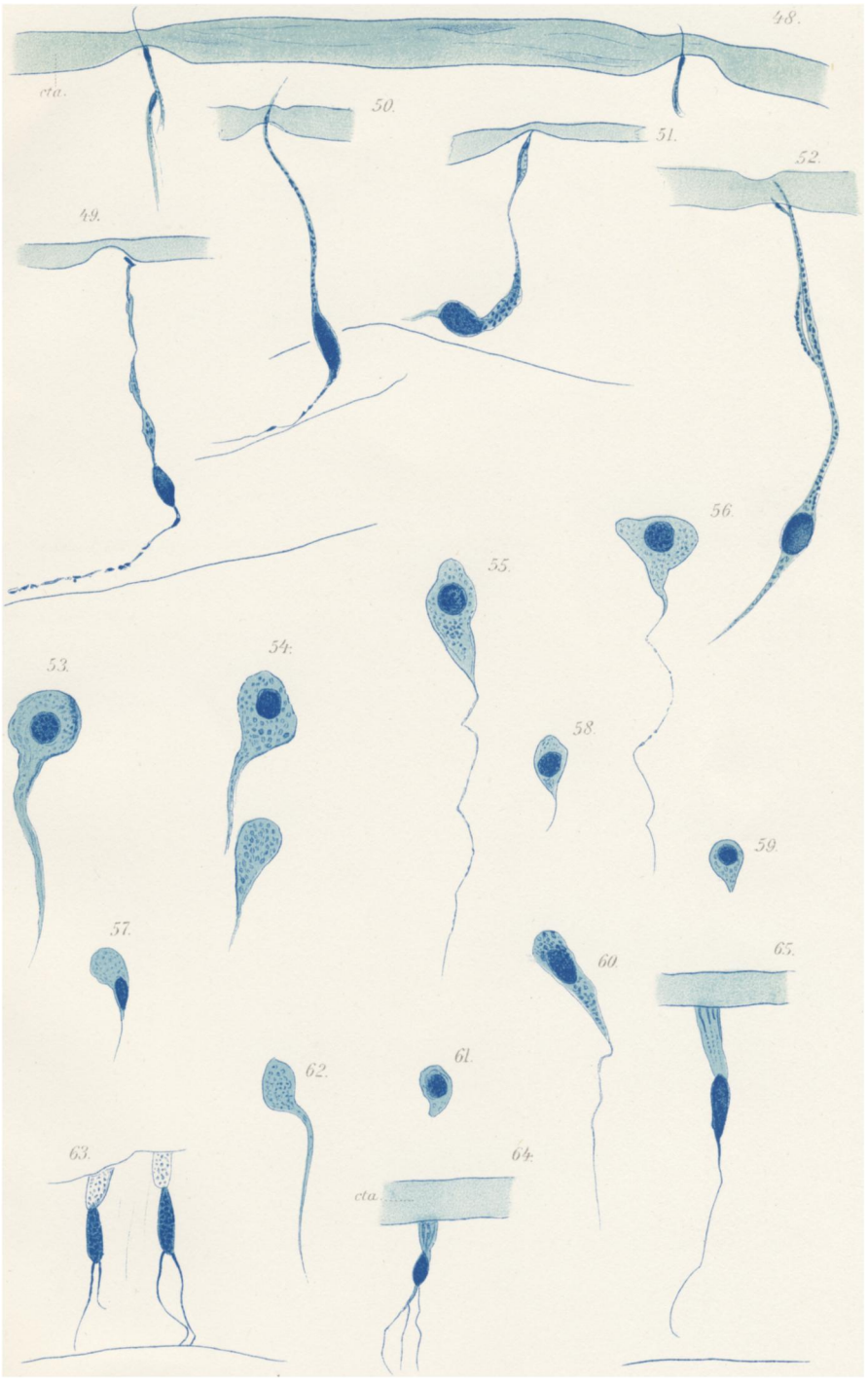

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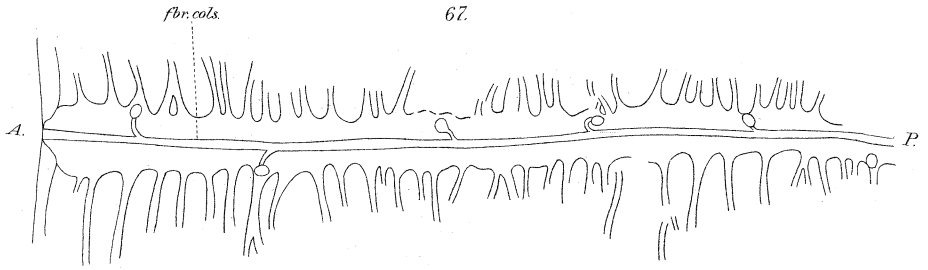
PLATE 8.

Figs. 66, 67. Diagrams of portions of nerve cord in *Clymene producta.*

Fig. 66. Nerve cord in the anterior part (about $\frac{2}{3}$) of segment 5, showing the lateral nerves, giant cells, and Leydig's fibre. × 40.

Fig. 67. Nerve cord in the anterior part (about $\frac{3}{4}$) of segment 3. × 40.

Fig 68. Diagram showing distribution of sense organs of right half of the first four segments of *Axiothea torquata.*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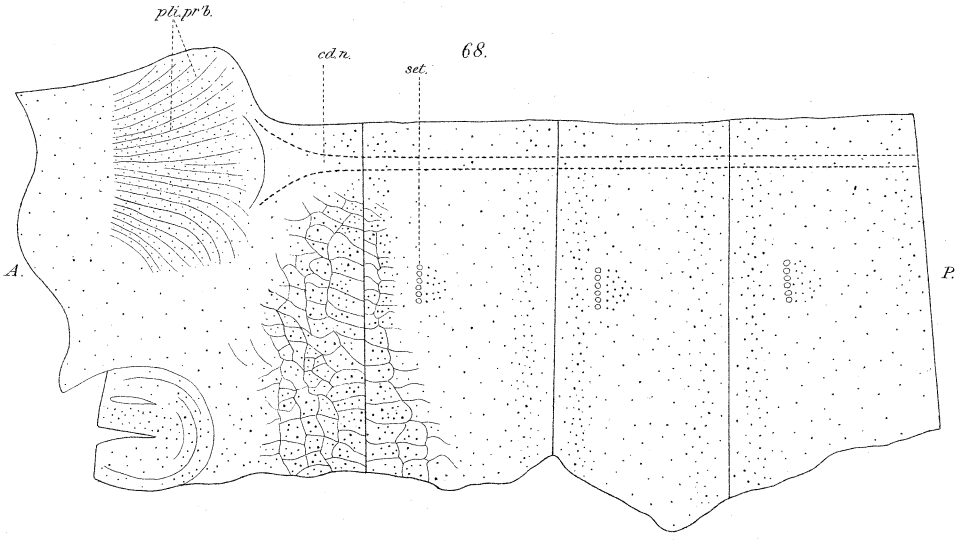
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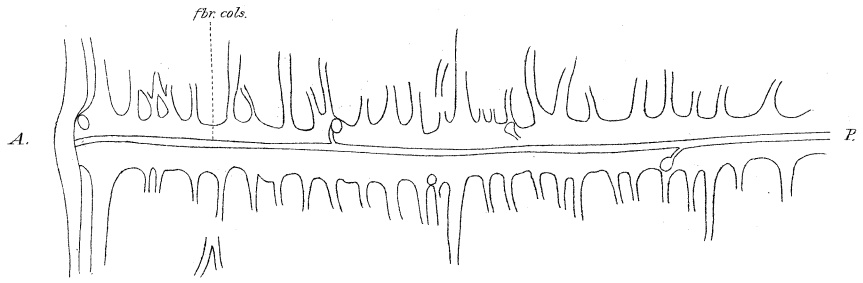
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